

to comprise an additional related pair (1253 and 1001) of around 40 kDa and a single spot (1119) of around 28 kDa. Because these two presumed proteins are present at substantially lower abundances than 413, and because the cytosolic HMG-CoA synthase is reported to consist of only one type of polypeptide, they are likely to represent other, very tightly coregulated enzymes. A second group of six spots was selected based on a regulatory pattern close to the inverse of that for spot 413 (MSN's 34, 79, 178, 182, 204, 347; data not shown). For these proteins, the lowest level of expression occurs with exposure to lovastatin plus cholestyramine and the highest level upon exposure to the high-cholesterol diet. Spots 182 and 79 are highly correlated and lie about one charge apart at the same molecular weight; they may thus be isoforms of a single protein. The other four spots probably represent additional enzymes or subunits.

3.3.2 MSN 235 and coregulated spots

A third group of five spots, mainly comprised of mitochondrial proteins including putative mitochondrial HMG-CoA synthase spots, showed a modest induction by lovastatin alone, but little or no effect with any of the other treatments (including the combination of lovastatin and cholestyramine; Fig. 12). This result is intriguing because lovastatin was expected to affect only the regulation of enzymes of cholesterol synthesis, which is entirely extra-mitochondrial. Three of the spots (235, 134, 144) form a closely-packed triad at approximately 30 kDa, and are likely to represent isoforms of one protein. All three spots are stained by an antibody to the mitochondrial form of HMG-CoA synthase obtained from Dr. Greenspan. Subcellular fractionation indicates a mitochondrial location. The other two spots (633 at about 38 kDa and 724 at about 69 kDa) are each present at lower abundance than the members of the triad.

3.3.3 An example of an anti-synergistic effect

A sixth spot (367) shows strong induction by lovastatin (two- to threefold), and about half as much induction with lovastatin plus cholestyramine, but without sharing the animal-animal heterogeneity pattern of the 235-set (Fig. 13). This protein is also mitochondrial, and represents the clearest example of an anti-synergistic effect of lovastatin and cholestyramine. The existence of such an effect demonstrates that lovastatin and cholestyramine do not act exclusively through the same regulatory pathway.

3.3.4 Complexity of the cholesterol synthesis pathway

Taken together, these results suggest that treatment with lovastatin alone can affect both cytosolic and mitochondrial pathways using HMG-CoA, while cholestyramine, on the other hand, either alone or in combination with lovastatin, produces a strong effect on the putative cytosolic pathway, but little or no effect on the putative mitochondrial pathway. An explanation for this difference may lie in lovastatin's effect on levels of HMG-CoA and related precursor compounds that are exchanged between the cytosol and the mitochondrion, whereas cholestyramine should affect only the cytosolic pathways directly controlled by cholesterol and bile acid levels. It remains to be explained why some

proteins of the putative mitochondrial pathway are so much more variable in their expression in all groups. An examination of all the coregulated groups suggests that quantitative statistical techniques can extract a wealth of interesting information from large sets of reproducible gels. The abundance of spots in the 413 coregulation group, for example, shows an amazing level of concordance in their relative expression among the five individuals of the lovastatin and cholestyramine treatment group. This effect is not due to differences in total protein loading, since they have already been removed by scaling, and since proteins with quite different regulation patterns can be demonstrated (e.g., Fig. 13). Such effects raise the possibility that many gene coregulation sets may be revealed through the study of a sufficiently large population of control animals (i.e., without any experimental manipulation). This approach, exploiting natural biological variation in protein expression instead of drug effects, offers an important incentive for the construction of a large library of control animal patterns.

4 Conclusions

Because of the widespread use of rat liver in both basic biochemistry and in toxicology, there is a long-term need for a comprehensive database of liver proteins. The rat liver master pattern presented here has proven to be an accurate representation of this system, having been matched to more than 700 gels to date. As the number of proteins identified and the number of compounds tested for gene expression effects grows, we expect this database to contribute valuable insights into gene regulation. Its practical utility in several areas of mechanistic toxicology is already being demonstrated.

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6 Addendum 1: Figures 1-13

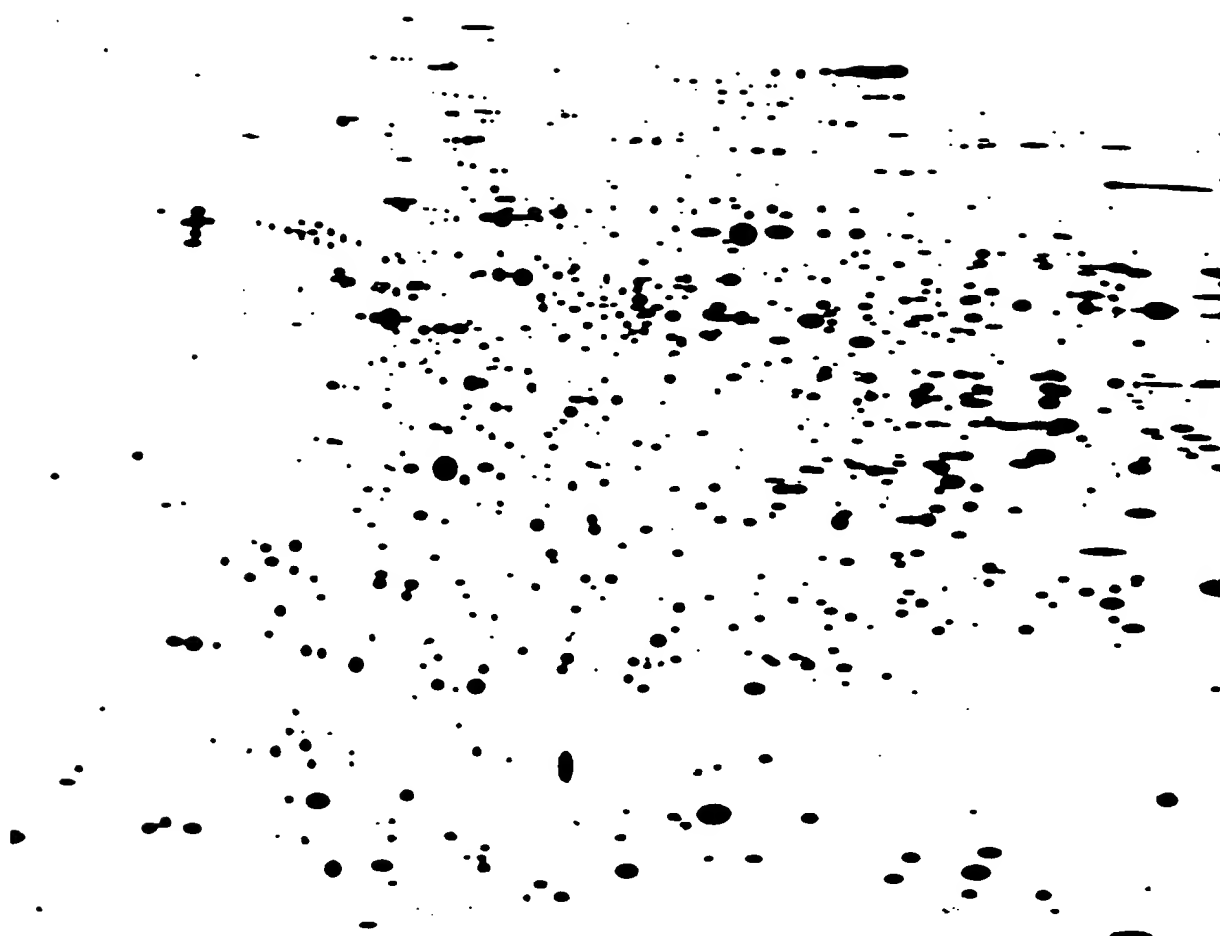


Figure 1. Synthetic representation of the standard rat liver 2-D master pattern, rendered as a greyscale image using a videoprinter.

2. Schem
ants.

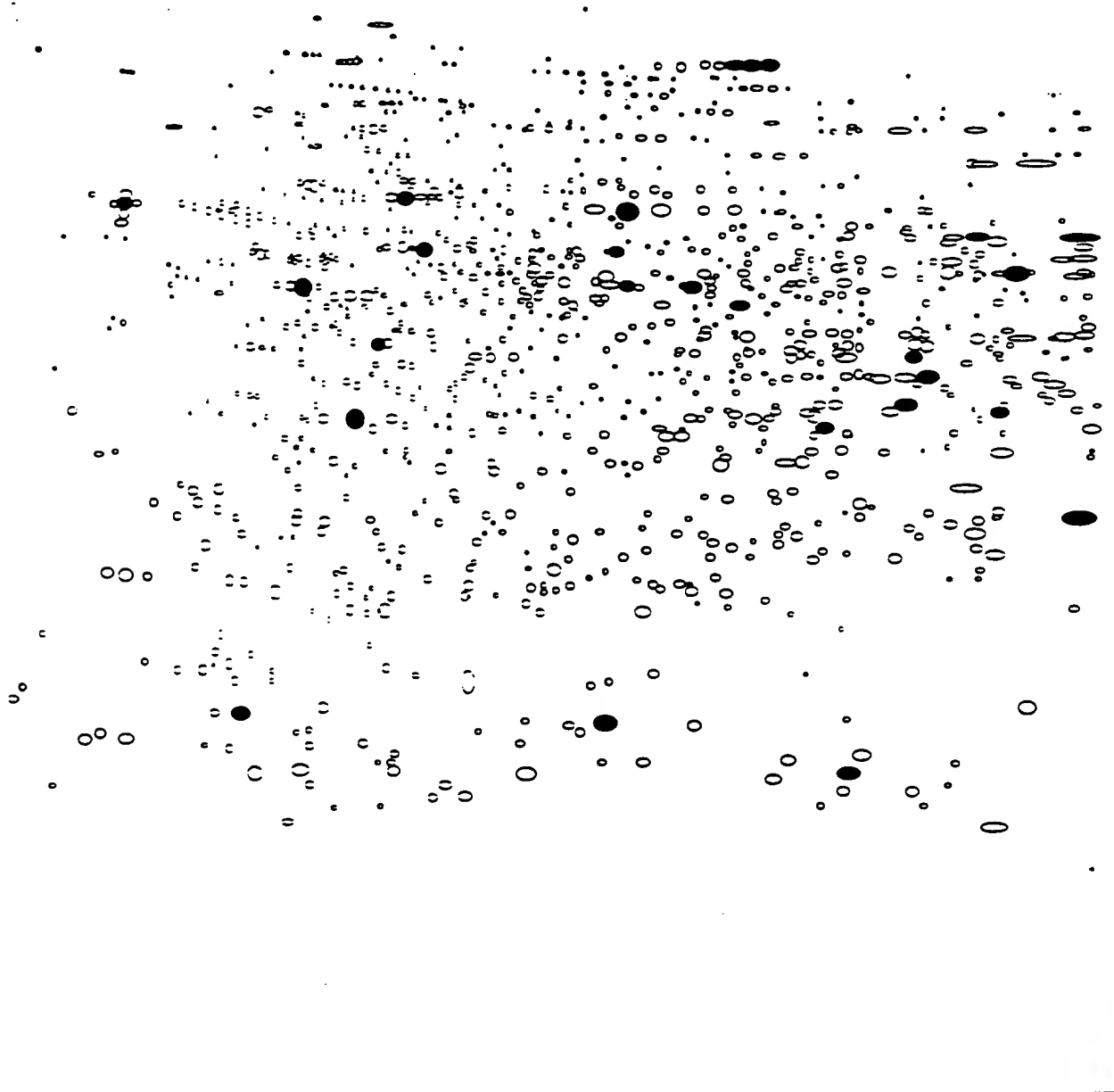


Fig. 2. Schematic representation of the master pattern (the same as Fig. 1), useful as an aid in relating specific areas of Fig. 1 and the following detailed fragments.

1

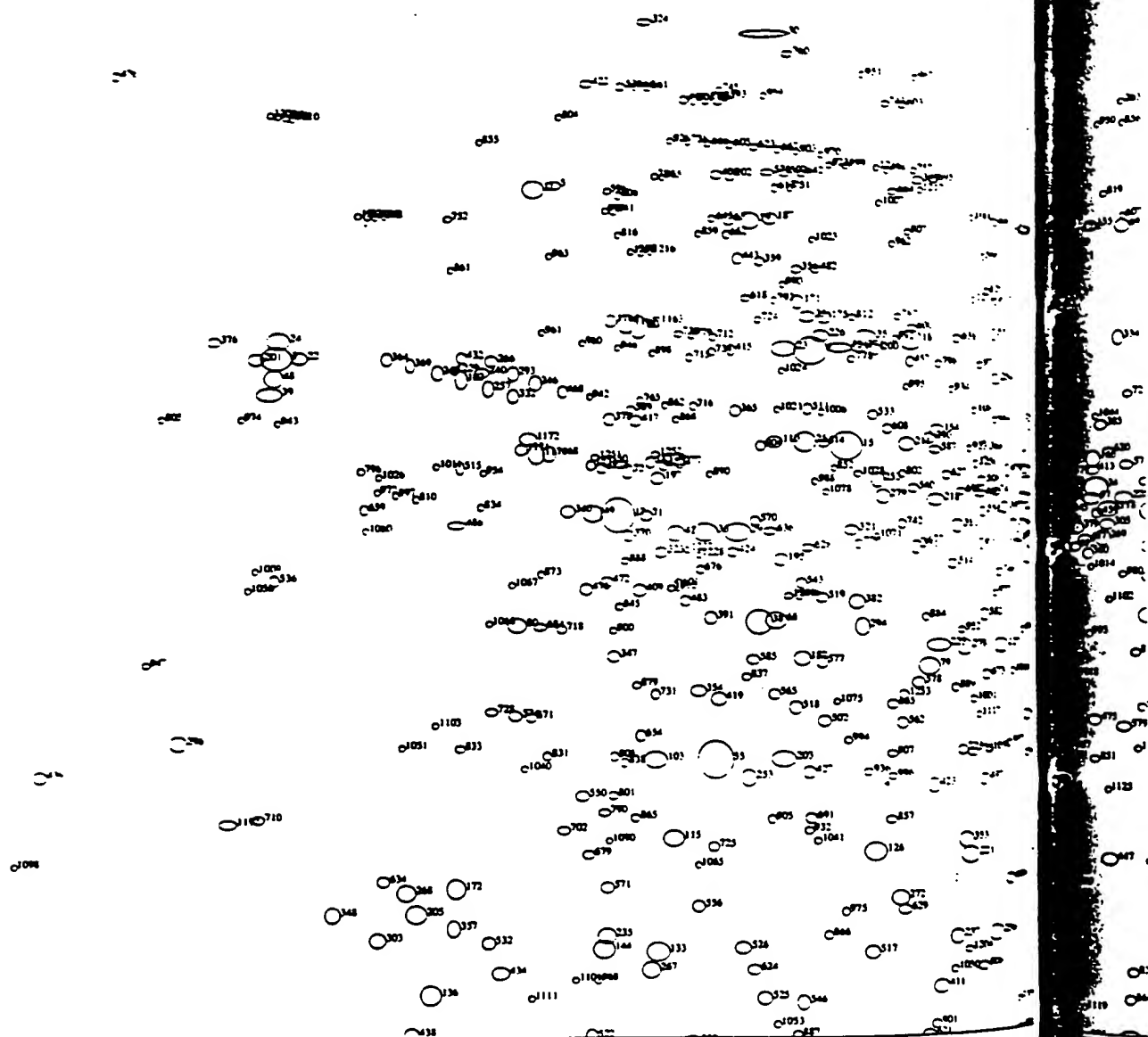


Figure 3. Upper left (high molecular weight, acidic) quadrant (#1) of the rat liver map, showing spot numbers.

4. Up

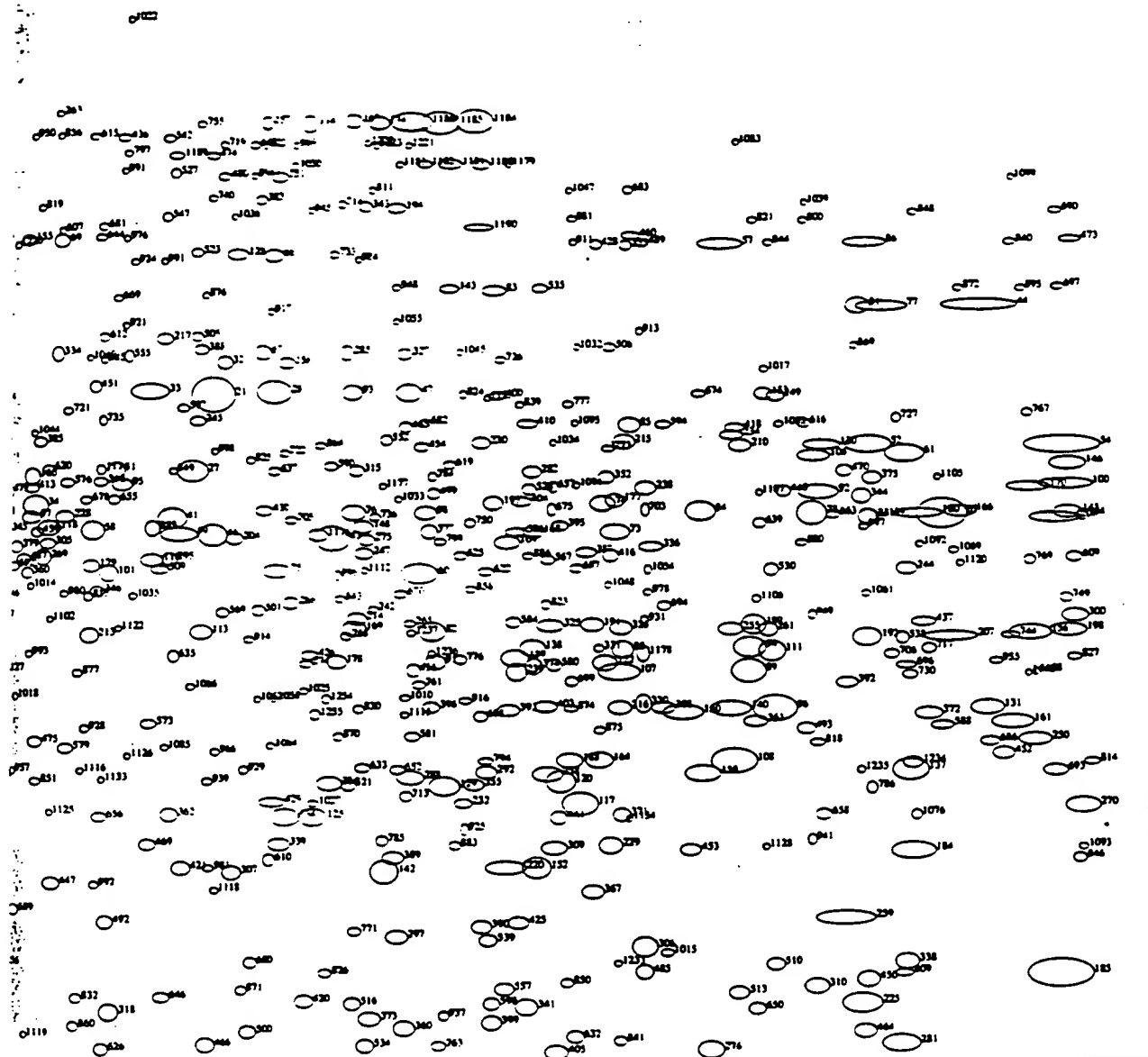
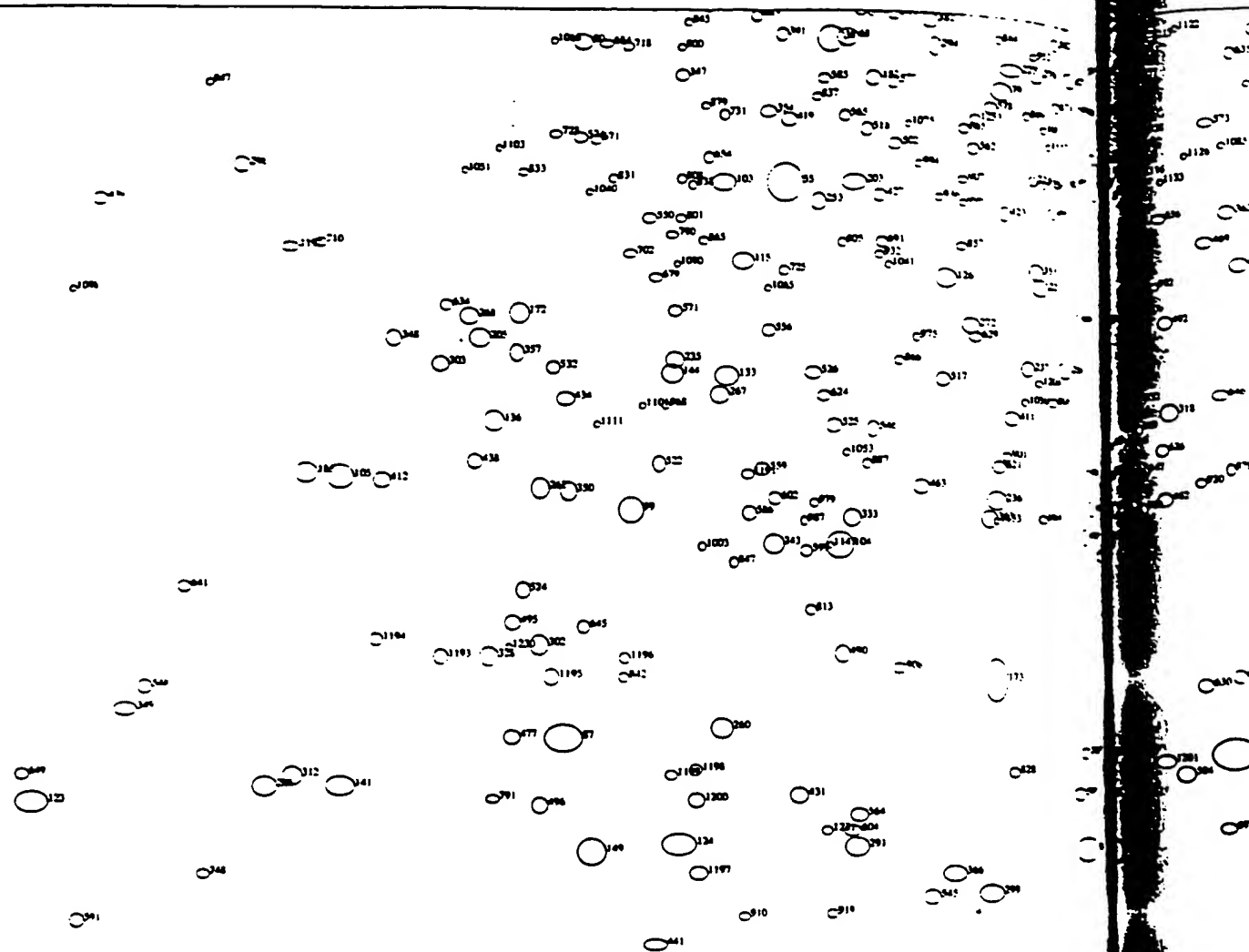


Figure 4. Upper right (high molecular weight, basic) quadrant (#2) of the rat liver map, showing spot numbers.



3

Figure 5. Lower left (low molecular weight, acidic) quadrant (#3) of the rat liver map, showing spot numbers.

Figure 6. Lower r

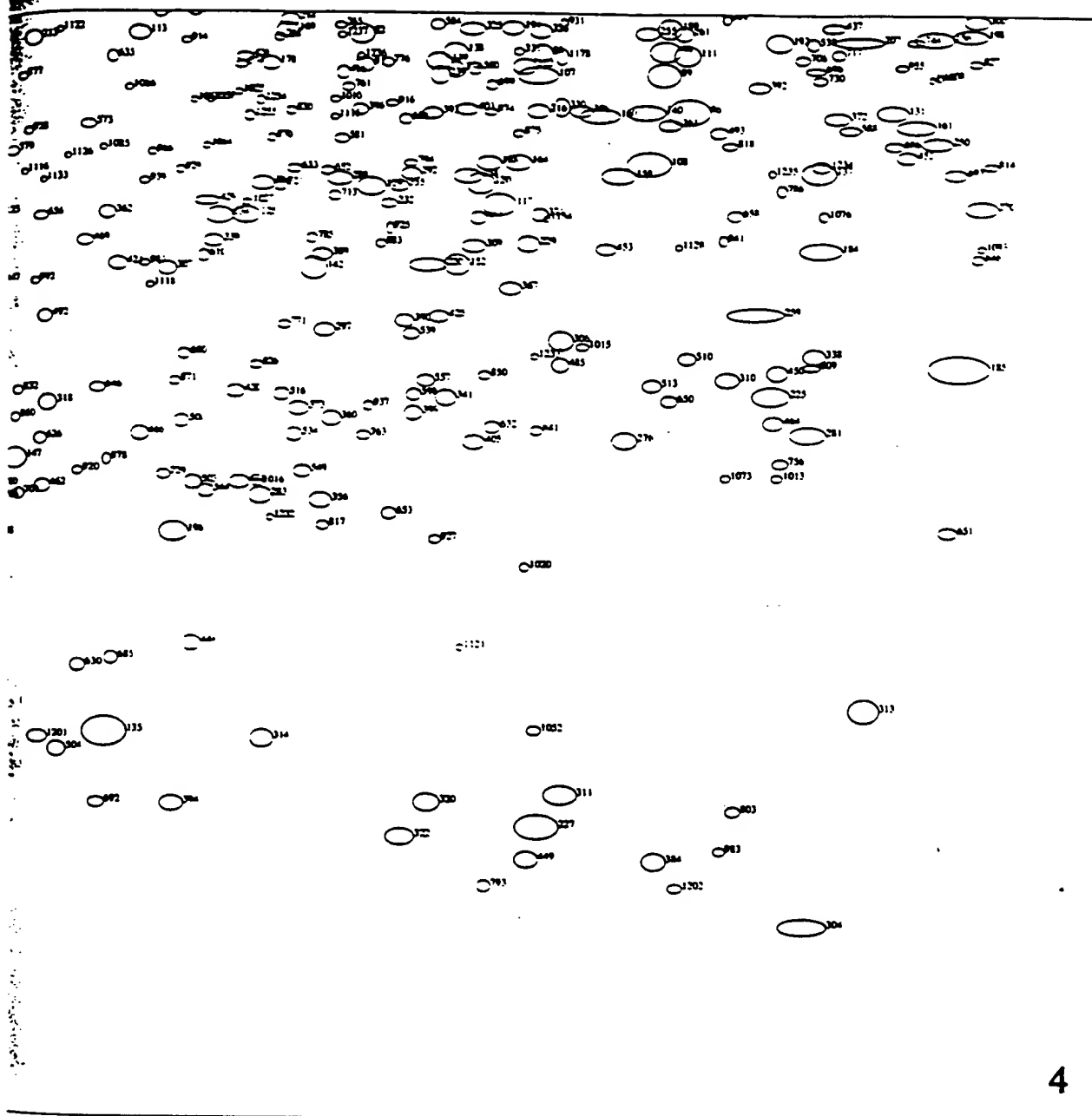
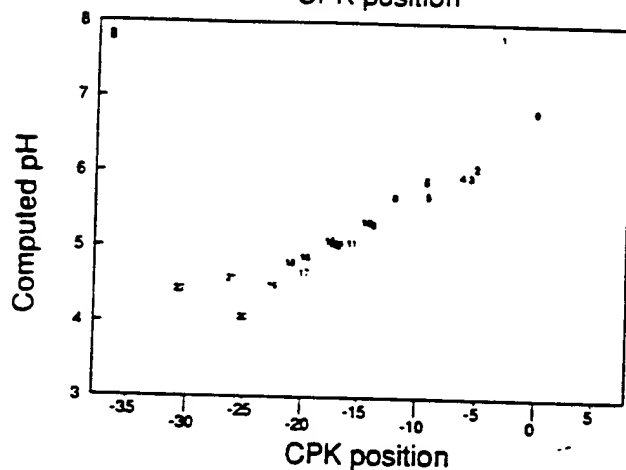
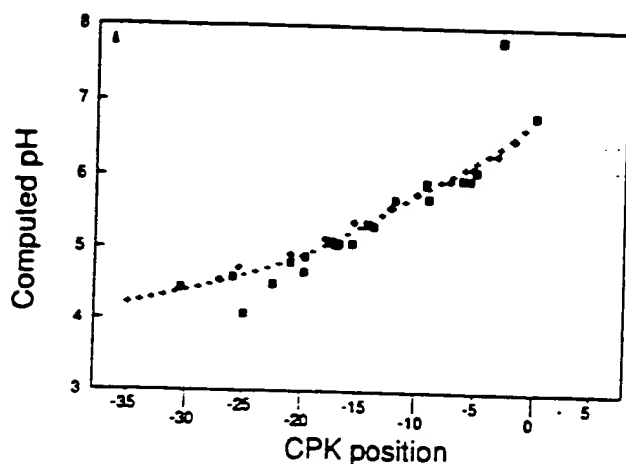
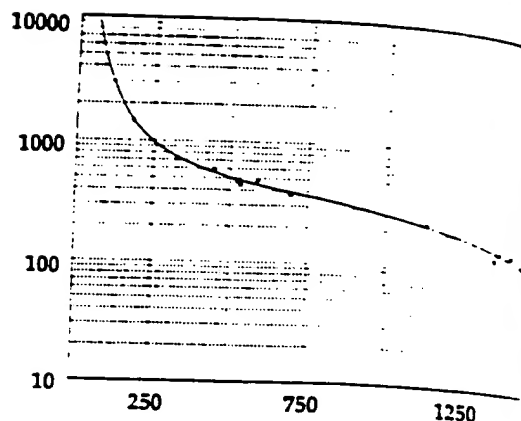


Figure 6. Lower right (low molecular weight, basic) quadrant (#4) of the rat liver map, showing spot numbers.



Number of Residues



Gel Y Coordinate

Figure 8. Plot of number of amino acids versus gel Y-position, with fitted curve used to predict molecular mass of unidentified proteins.

Figure 7. (a) Plot of computed isoelectric point versus gel X-position for two sets of carbamylated standard proteins (rabbit muscle CPK [—] and human hemoglobin β chain, filled diamonds) and several other proteins (shaded squares). (b) The identities of the various proteins represented by the squares are indicated by the numbers in corresponding positions on (a); these refer to Table 4.

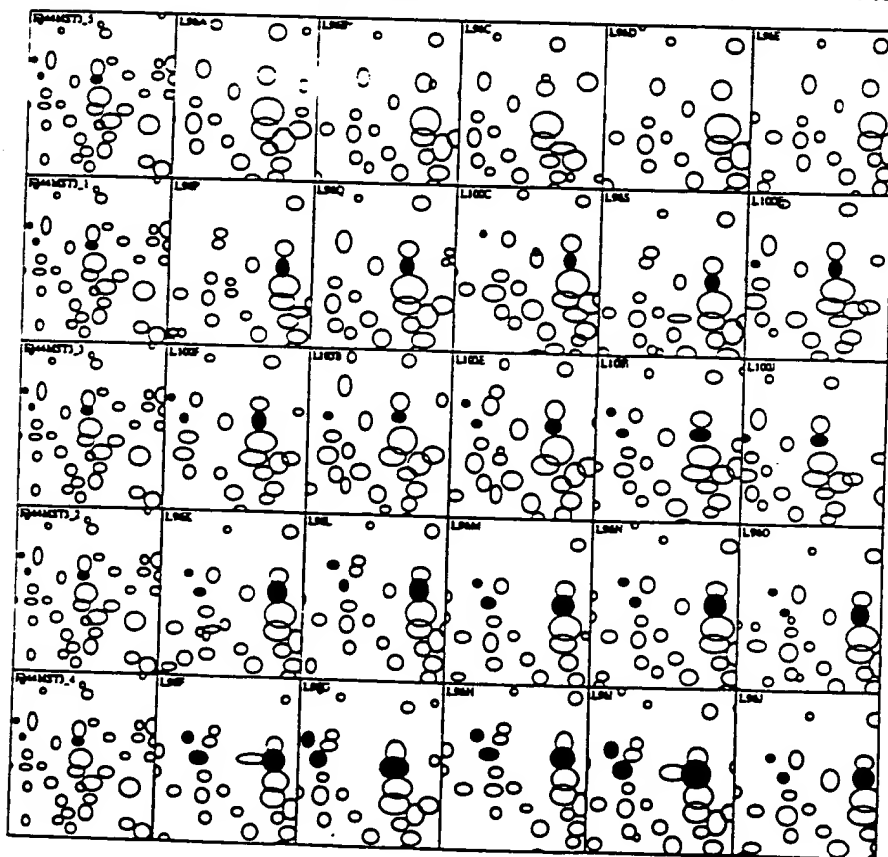


Figure 9. Montage showing effects in the region of MSN:413. The montage shows a small window into one portion of the 2-D pattern, one row of windows for each experimental group, and one panel for each gel in the experiment. The left-most pattern in each row is a group-specific copy of the master pattern followed by the patterns for the five individual rats in the group. The highlighted protein spots (filled circles) are spot 413 (on the right of each panel; identified as cytosolic HMG-CoA synthase) and two modified forms of it (1250 and 933). From the top, the rows (experimental groups) are: high cholesterol, controls, cholestyramine, lovastatin, and lovastatin plus cholestyramine.

Regulation of Rat Liver 413

(Putative Cytosolic HMG-CoA Synthase, 53kd)
Test Compounds in Diet

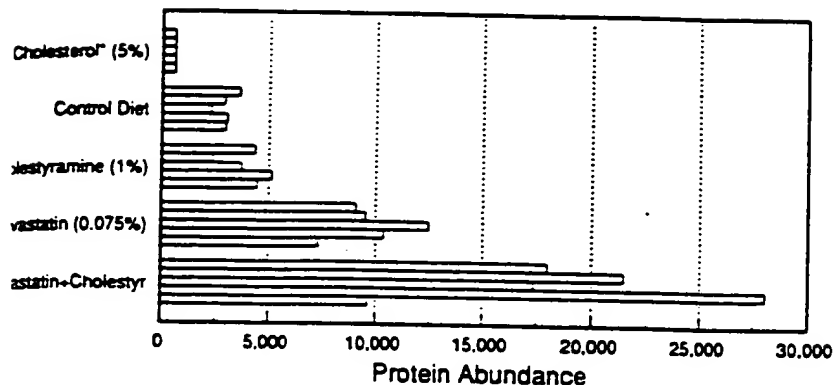


Figure 10. Bargraph showing the quantitative effects of various treatments on the abundance of MSN:413 (cytosolic HMG-CoA synthase) in the gels of Fig. 9.

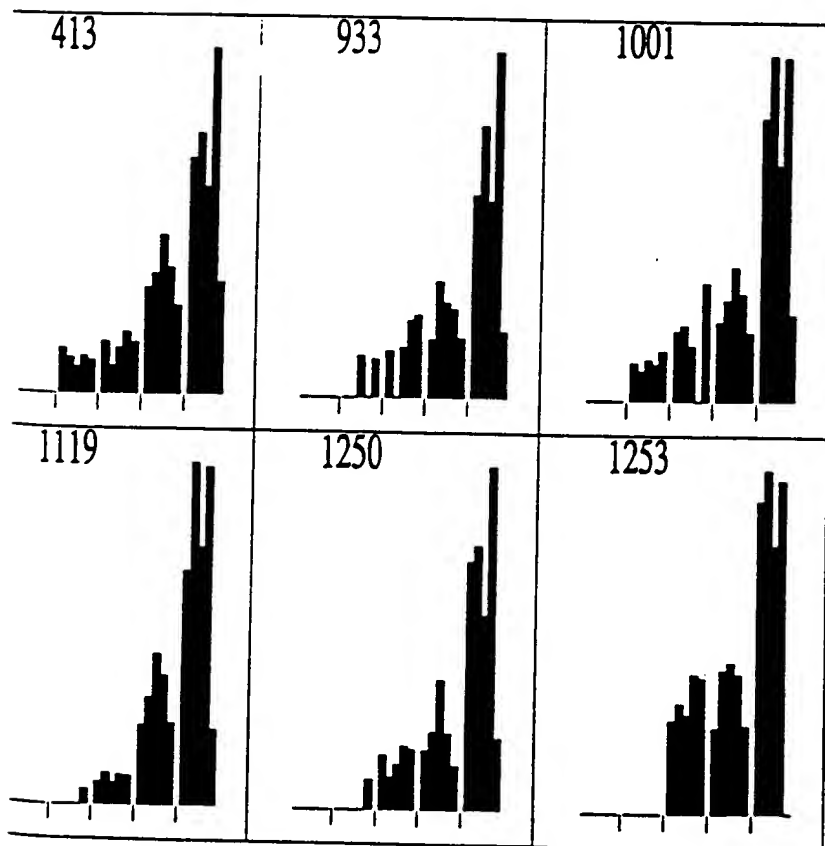


Figure 11. Bargraphs of a series of six coregulated spots including MSN:413. In the bargraphs, the abundances of the appropriate spot (master spot number shown at the top of the panel) in each animal are shown. The five five-animal groups are in the order (left to right): high cholesterol, controls, cholestyramine, lovastatin, and lovastatin plus cholestyramine. Each bar within a group represents one experimental animal liver (one 2-D gel). Note the correlated expression of the 6 spots, especially in the two far right (most strongly induced) groups.

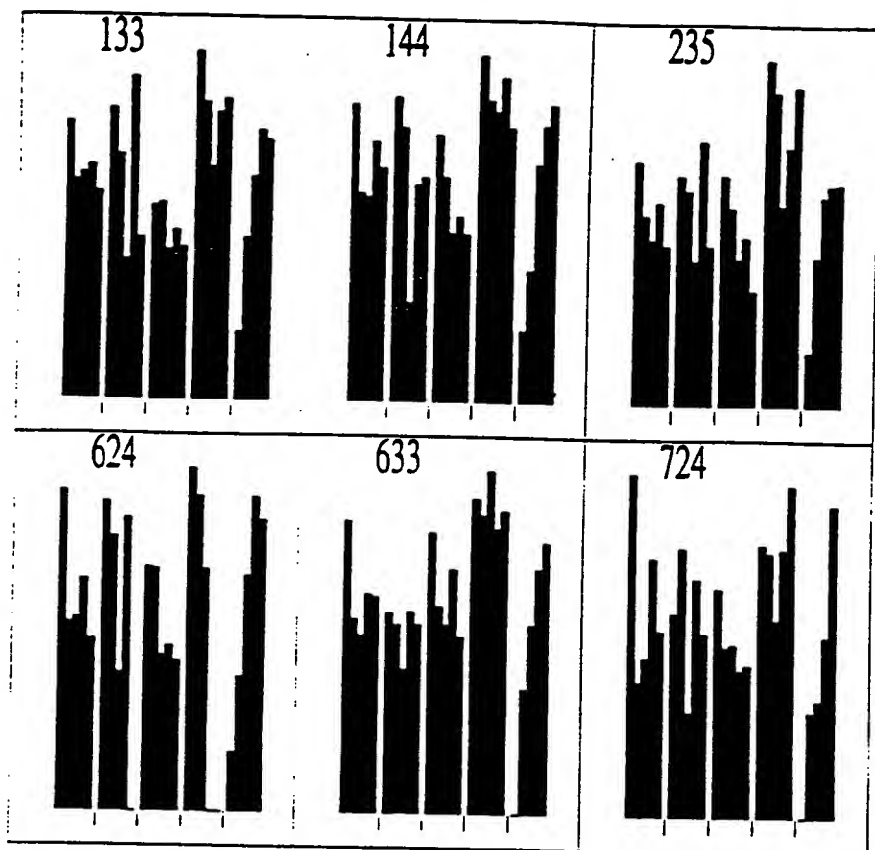


Figure 12. Data on a second coregulated group of spots, presented as in Fig. 11. The fourth experimental group (lovastatin) shows a modest induction, while the fifth group (lovastatin plus cholestyramine) does not.

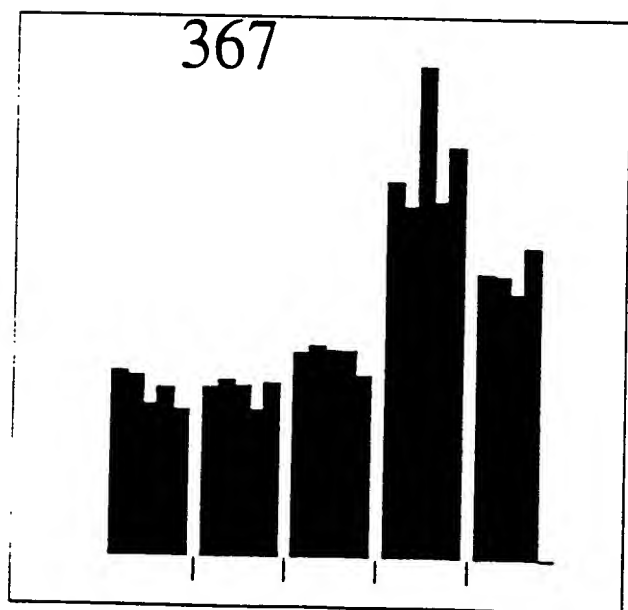


Figure 13. Data on spot MSN:367, presented as in Fig. 11. This protein shows unambiguously the anti-synergistic effect of lovastatin and cholestyramine (fifth group) as compared to lovastatin (fourth group). This response contrasts strongly with the regulation pattern seen in Fig. 11.

Mass	x
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4908	
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Inter table of:
Predicted spots

Table 1. Master table of proteins in the rat liver database^{a1}

MSN	X	Y	CPK ₀₁	SOSMW	MSN	X	Y	CPK ₀₁	SOSMW	MSN	X	Y	CPK ₀₁	SOSMW
3	311	434	<-35.0	63,800	85	1119	536	-9.9	53,800	174	1364	183	-6.7	162,900
5	568	263	-24.3	102,900	86	1731	756	-2.0	40,700	175	825	393	-15.7	69,300
8	812	426	-16.0	64,800	97	1033	566	-11.4	51,600	177	1582	553	-3.6	52,600
11	549	268	-25.2	101,000	98	1406	565	-6.1	51,700	178	1321	710	-7.2	43,000
15	845	520	-15.3	55,200	99	578	1149	-23.8	25,000	179	1089	615	-10.4	48,300
17	629	589	-21.6	50,000	100	2004	538	>0.0	53,700	180	1866	567	-0.5	51,600
18	906	414	-14.0	66,300	101	1106	623	-10.1	47,800	181	411	295	-32.1	91,200
19	755	298	-17.5	90,200	102	482	455	-28.5	61,300	182	804	730	-16.2	42,000
20	649	403	-20.9	67,900	103	665	830	-20.2	37,300	184	1860	896	-0.6	34,500
21	1204	448	-8.7	62,100	104	773	1182	-17.0	23,800	185	1997	1017	>0.0	29,800
22	332	434	<-35.0	63,800	105	312	1117	<-35.0	26,100	186	279	1113	<-35.0	26,300
23	787	424	-16.6	65,000	106	1769	509	-1.5	56,100	187	773	296	-17.0	90,800
24	313	417	<-35.0	66,000	107	1585	720	-3.6	42,500	188	1538	807	-4.2	38,400
25	807	516	-16.1	55,500	108	1692	807	-2.4	38,300	191	1560	674	-3.9	44,900
27	1184	524	-9.0	54,800	109	1482	583	-4.8	49,700	192	1818	687	-0.9	44,200
28	1263	446	-8.0	62,400	110	778	516	-16.9	55,500	193	1469	555	-5.0	52,400
29	743	605	-17.8	49,000	111	1728	700	-2.0	43,500	194	1380	266	-6.4	101,600
30	768	112	-17.2	348,600	113	1191	680	-8.9	44,500	195	784	632	-16.7	47,300
32	1216	417	-8.6	66,000	114	1298	185	-7.5	160,800	196	1227	1185	-8.4	23,700
33	1145	445	-9.5	62,500	115	682	907	-19.6	34,100	197	667	553	-20.1	52,600
34	1037	555	-11.3	52,400	116	1146	610	-9.5	48,700	198	2006	681	>0.0	44,500
35	863	412	-14.9	66,600	117	1548	849	-4.1	36,500	199	1711	674	-2.2	44,900
36	712	606	-18.7	48,900	118	1050	577	-11.1	50,800	200	872	424	-14.7	65,000
38	763	694	-17.3	43,800	120	1530	828	-4.3	37,400	201	292	435	<-35.0	63,700
39	304	470	<-35.0	59,800	121	838	423	-15.4	65,200	202	736	253	-18.0	107,800
41	1165	569	-9.2	51,400	122	1572	712	-3.8	42,900	203	786	829	-16.7	37,400
42	684	607	-19.6	48,800	123	23	1433	<-35.0	15,300	204	1224	589	-8.5	50,000
43	1318	589	-7.3	50,000	124	621	1474	-21.9	13,900	205	439	983	-30.9	31,100
44	1924	362	-0.1	74,600	125	1298	862	-7.5	36,000	206	1994	571	>0.0	51,300
46	1203	586	-8.7	50,200	126	872	921	-14.7	33,500	207	1895	687	-0.3	44,200
47	1391	447	-6.3	62,300	127	1000	717	-12.0	42,600	208	240	1418	<-35.0	15,800
48	309	454	<-35.0	61,500	128	1229	311	-8.4	86,100	210	1700	499	-2.3	57,000
49	605	587	-22.5	50,100	129	1422	832	-5.8	37,300	211	902	517	-14.1	55,400
50	621	535	-21.8	53,900	130	1776	499	-1.4	57,000	213	1087	684	-10.4	44,400
51	1113	522	-10.0	55,000	131	1930	757	-0.1	40,700	214	1340	668	-7.0	45,200
52	1820	499	-0.9	57,000	132	660	537	-20.4	53,800	215	1591	495	-3.5	57,300
53	725	177	-18.3	170,800	133	666	1019	-20.2	29,700	216	1585	755	-3.6	40,700
54	2001	500	>0.0	56,900	134	1271	862	-7.9	36,000	217	1159	393	-9.3	69,300
55	722	830	-18.4	37,300	135	1161	1389	-9.3	16,800	218	931	572	-13.5	51,200
56	678	533	-19.8	54,100	136	453	1063	-29.7	28,100	219	713	177	-18.7	170,500
57	1682	302	-2.5	89,000	137	1858	823	-0.6	37,700	220	1479	911	-4.9	33,900
58	1091	580	-10.3	50,600	138	1504	697	-4.6	43,700	221	965	927	-12.8	33,300
59	1171	585	-9.2	50,300	139	1488	707	-4.8	43,200	223	934	716	-13.5	42,700
60	1400	624	-6.2	47,800	140	1689	756	-2.4	40,700	225	1812	1045	-1.0	28,800
61	1853	508	-0.6	56,200	141	311	1417	<-35.0	15,800	226	821	411	-15.8	66,800
62	1888	567	-0.4	51,500	142	1366	915	-6.7	33,800	227	1586	1483	-3.6	13,600
65	735	297	-18.1	90,500	143	1429	346	-5.7	77,900	228	1065	567	-10.8	51,600
66	1263	312	-8.0	85,900	144	615	1017	-22.1	29,800	229	1577	890	-3.7	34,800
67	1252	407	-8.1	67,300	145	2006	566	>0.0	51,600	230	1458	496	-5.2	57,300
68	779	692	-16.8	43,900	146	2006	518	>0.0	55,300	232	1440	849	-5.5	36,500
69	1064	296	-10.8	90,800	147	1070	1108	-10.7	26,500	234	1692	489	-2.4	57,900
71	656	589	-20.6	50,000	148	1347	578	-6.9	50,800	235	618	1004	-22.0	30,300
72	638	545	-21.2	53,100	149	541	1481	-25.7	13,700	236	920	1138	-13.7	25,400
73	1582	583	-3.6	50,400	150	1645	760	-2.8	40,500	237	952	1008	-13.1	30,200
74	1570	556	-3.8	52,300	151	1269	236	-7.9	117,000	238	1611	541	-3.2	53,500
75	1264	621	-8.0	48,000	152	1507	911	-4.5	33,900	239	1489	720	-4.8	42,500
76	1338	564	-7.0	51,800	153	1722	448	-2.1	62,100	240	501	448	-27.7	62,100
77	1833	363	-0.8	74,400	154	932	503	-13.5	56,600	241	1820	569	-0.9	51,400
78	1767	565	-1.5	51,700	155	1031	294	-11.4	91,400	242	1357	658	-6.8	45,800
79	925	738	-13.6	41,600	156	1970	684	>0.0	44,400	243	711	1182	-18.7	23,800
80	534	698	-26.1	43,600	157	1258	183	-8.1	162,400	244	1855	621	-0.6	48,000
81	1811	363	-1.0	74,500	158	1275	417	-7.8	65,900	245	1189	474	-8.9	59,300
82	1412	681	-6.0	44,500	159	1663	820	-2.6	37,800	246	551	459	-25.1	61,000
83	1471	347	-5.0	77,500	160	1034	527	-11.4	54,600	247	1348	604	-6.9	49,100
84	1662	563	-2.7	51,800	161	1953	771	>0.0	40,000	248	460	448	-29.3	62,100
85	1596	479	-3.4	58,900	162	1020	1482	-11.6	13,700	249	1733	451	-1.9	61,800
86	1817	301	-0.9	89,100	164	1566	806	-3.8	38,400	250	1974	788	>0.0	39,200
87	516	1371	-27.0	17,400	166	1905	565	-0.2	51,700	251	808	392	-16.1	69,500
88	1589	698	-3.5	43,600	167	1340	181	-7.0	164,900	252	874	553	-14.6	52,500
89	1706	719	-2.2	42,500	168	1506	583	-4.6	50,400	253	753	848	-17.6	36,500
90	651	329	-20.8	81,700	169	1338	678	-7.0	44,700	254	995	450	-12.1	61,900
91	1415	710	-6.0	43,000	170	1969	541	>0.0	53,500	255	1690	679	-2.4	44,600
92	1773	545	-1.4	53,200	171	800	378	-16.3	71,800	256	994	1006	-12.1	30,200
93	1338	446	-7.0	62,300	172	476	958	-28.7	32,100	257	508	464	-27.4	60,400
94	1708	696	-2.2	43,700	173	919	1314	-13.7	19,300	258	1517	820	-4.4	37,800

^{a1} Master table of proteins in the rat liver database, showing spot master number, gel position (x and y), isoelectric point relative to CPK standards, and predicted molecular mass (from the standard curve of Fig. 8).

MSN	X	Y	CPKoi	SOSMW	MSN	X	Y	CPKoi	SOSMW	MSN	X	Y	CPKoi	SOSMW	MSN	X
259	1796	961	-1.1	31,900	345	1006	578	-11.9	50,800	426	1296	704	-7.6	43,300	809	
260	661	1361	-20.4	17,700	346	1095	640	-10.3	46,800	427	810	843	-16.0	36,800	1099	
261	1725	679	-2.0	44,800	347	625	728	-21.7	42,000	428	1565	303	-3.9	88,700	1696	
262	486	1127	-28.0	25,800	348	361	983	-35.3	31,100	429	1259	847	-8.0	36,800	948	
263	1063	172	-10.9	177,400	349	110	1343	<-35.0	18,300	430	1253	562	-8.1	51,900	481	
265	1390	673	-6.3	45,000	350	521	1130	-26.7	25,700	431	734	1426	-18.1	15,900	1334	
266	510	437	-27.3	63,400	351	912	619	-13.9	48,100	432	483	433	-28.5	63,900	868	
267	660	1038	-20.4	29,000	352	1574	530	-3.7	54,300	434	518	1041	-26.9	28,900	798	
268	430	961	-31.0	31,900	353	961	912	-12.9	33,900	435	1020	1170	-11.6	24,300	822	
269	1044	606	-11.2	48,900	354	706	762	-18.9	40,400	436	1122	196	-9.8	147,800	632	
270	2019	853	>0.0	36,300	355	1450	830	-5.3	37,300	437	1870	673	-0.5	45,000	1332	
271	857	422	-15.0	65,200	356	1374	1152	-6.5	24,900	438	435	1102	-31.0	26,700	803	
272	895	968	-14.2	31,700	357	474	997	-28.7	30,600	439	86	847	<-35.0	36,800	1190	
274	1292	712	-7.6	42,900	358	798	346	-16.3	77,800	440	1740	544	-1.8	53,200	479	
275	1350	590	-6.9	49,900	359	764	338	-17.3	79,400	441	599	1571	-22.8	10,800	768	
276	1670	1089	-2.6	27,100	360	1384	1068	-6.4	27,900	443	743	335	-17.8	80,100	747	
277	688	538	-19.4	53,700	361	1713	769	-2.1	40,100	446	801	668	-16.2	45,200	1170	
278	961	718	-13.0	42,600	362	1161	859	-9.3	36,100	447	1050	926	-11.1	33,300	1502	
279	879	570	-14.5	51,300	363	914	1156	-13.8	24,800	448	1245	1298	-8.2	19,800	1728	
281	1848	1084	-0.7	27,300	364	412	435	-32.0	63,700	449	1576	1516	-3.7	12,600	507	
282	1505	525	-4.6	54,800	365	741	486	-17.9	58,200	450	1818	1021	-0.9	29,800	870	
283	1313	1147	-7.3	25,100	366	878	1503	-14.6	13,000	451	1094	440	-10.3	63,100	1347	
284	1314	829	-7.3	37,400	367	1560	935	-3.9	33,000	452	1945	802	>0.0	38,800	1513	
285	1332	408	-7.1	67,200	368	983	520	-12.4	55,200	453	1652	894	-2.8	34,800	308	
286	1277	652	-7.8	46,100	369	434	441	-31.0	63,000	454	1403	500	-6.1	56,900	1851	
288	1391	824	-6.3	37,600	370	639	610	-21.2	48,700	456	1394	718	-6.3	42,800	1483	
289	1147	579	-9.5	50,700	371	1587	860	-3.6	36,100	457	905	436	-14.0	63,500	909	
290	925	511	-13.6	55,900	372	1875	762	-0.5	40,400	459	1038	581	-11.3	50,500	625	
291	787	1476	-16.6	13,900	373	1351	1059	-6.8	28,300	460	1598	294	-3.4	91,400	1164	
292	1462	818	-5.1	37,800	374	1506	715	-4.6	42,700	461	1528	863	-4.3	35,900	803	
293	531	449	-26.3	62,000	375	1823	532	-0.9	54,200	462	1098	1137	-10.2	25,400	1259	
294	860	698	-14.9	43,600	376	254	417	<-35.0	65,900	463	849	1125	-15.2	25,800	856	
295	1162	609	-9.3	48,700	377	1409	583	-6.1	50,400	464	1814	1072	-0.9	27,800	803	
296	218	814	<-35.0	38,000	378	621	494	-21.8	57,500	465	1388	481	-6.3	58,700	1182	
297	1377	979	-6.5	31,300	379	1017	595	-11.7	49,600	466	1194	1084	-8.9	27,300	128	
299	913	1523	-13.9	12,400	381	953	598	-13.1	49,400	468	577	467	-23.9	60,100	1355	
300	2012	667	>0.0	45,300	382	856	674	-15.0	44,900	469	1140	888	-9.6	34,900	595	
301	702	178	-19.0	169,200	383	1252	258	-8.1	105,300	470	1797	524	-1.1	54,800	1369	
302	494	1280	-28.1	20,400	384	1699	1518	-2.3	12,500	471	1293	1133	-7.6	25,500	982	
303	403	1008	-32.6	30,100	385	1042	493	-11.2	57,500	472	618	655	-21.9	46,000	1125	
304	1843	1585	-0.7	10,300	386	1490	583	-4.7	50,400	473	2009	299	>0.0	89,900	705	
305	1049	593	-11.1	49,800	387	1554	603	-4.0	49,100	474	1205	215	-8.7	131,300	1477	
306	1608	989	-3.3	30,900	388	1193	404	-8.9	67,700	475	1035	788	-11.4	39,200	980	
307	1219	916	-8.5	33,700	389	1374	902	-6.5	34,300	476	160	155	<-35.0	207,800	700	
308	1627	755	-3.0	40,700	390	1456	969	-5.2	31,700	477	469	1370	-28.9	17,400	1028	
309	1524	892	-4.4	34,700	391	718	690	-18.5	44,000	478	599	662	-22.8	45,800	808	
310	1789	1028	-1.5	29,400	392	1799	732	-1.1	41,900	479	1009	540	-11.8	53,500	789	
311	1609	1451	-3.3	14,700	393	1482	758	-4.8	40,600	480	1216	235	-8.6	117,400	777	
312	266	1408	<-35.0	16,100	394	1227	1461	-8.4	14,400	482	816	346	-15.9	77,800	980	
313	1902	1365	-0.3	17,600	395	1530	577	-4.3	50,800	483	693	673	-19.3	44,900	1519	
314	1316	1395	-7.3	16,600	396	1410	755	-6.0	40,800	485	1608	1013	-3.3	30,000	1212	
315	1341	523	-7.0	54,900	397	912	256	-13.9	106,400	486	478	599	-28.6	49,300	760	
318	1104	1053	-10.1	28,500	399	1465	1063	-5.0	28,100	487	1025	607	-11.5	48,800	818	
320	1480	1459	-4.9	14,400	400	1473	450	-4.9	61,900	488	1045	1186	-11.2	23,700	1142	
321	850	603	-15.1	49,100	401	1029	1140	-11.5	25,300	489	1609	301	-3.3	89,200	532	
322	1454	1494	-5.3	13,300	403	1516	754	-4.4	40,800	490	775	1289	-17.0	20,100	771	
323	670	626	-20.0	47,700	404	1495	554	-4.7	52,500	491	692	178	-19.3	169,300	1068	
324	655	101	-20.6	420,500	405	1525	1092	-4.3	27,100	492	1100	964	-10.2	31,800	822	
325	1521	675	-4.4	44,800	406	723	252	-18.4	108,000	493	1760	776	-1.6	39,700	914	
326	1587	677	-3.6	44,700	408	650	663	-20.8	45,500	494	882	247	-14.5	110,700	1084	
327	1388	409	-6.3	67,000	410	1501	478	-4.6	59,000	495	470	1258	-28.9	21,200	1524	
328	448	1291	-30.0	20,100	411	836	1057	-13.4	28,300	496	494	1436	-28.1	15,200	1392	
330	1808	751	-3.3	40,900	412	350	1120	-35.9	26,000	497	980	852	-12.5	36,400	982	
331	1566	687	-3.8	43,700	413	1033	538	-11.4	53,700	499	1414	546	-6.0	53,100	1487	
332	531	471	-26.3	59,600	415	737	425	-18.0	64,900	500	1234	1072	-8.3	27,800	758	
333	784	1156	-16.7	24,700	416	1578	606	-3.7	48,900	501	1246	659	-8.2	45,700	687	
334	1059	407	-10.9	67,300	417	646	496	-21.0	57,300	502	824	792	-15.7	39,000	830	
335	1593	303	-3.5	88,500	418	1695	482	-2.3	58,600	503	1246	1134	-8.2	25,500	1888	
336	1616	598	-3.2	49,400	419	725	770	-18.3	40,000	504	1115	1407	-9.9	16,200	842	
338	1854	1004	-0.6	30,300	420	1289	1041	-7.7	28,900	505	1189	391	-8.9	68,700	1317	
339	1265	888	-8.0	34,900	421	1171	912	-9.1	33,900	506	1578	402	-3.7	68,000	85	
340	581	585	-23.6	50,300	422	599	162	-22.8	193,700	507	787	250	-16.6	109,000	1014	
341	1497	1047	-4.7	28,700	423	929	856	-13.6	36,200	508	979	552	-12.5	52,800	732	
343	1351	265	-6.8	102,200	424	739	625	-17.9	47,700	509	1153	619	-9.4	48,100	1827	
344	1813	549	-0.9	52,800	425	1490	965	-4.7	31,800	510	1730	1006	-2.0	30,200	1009	

SN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW
511	809	484	-16.0	58,400	596	619	269	-21.9	100,500	674	1661	448	-2.7	62,100
512	1099	533	-10.2	54,100	597	1176	461	-9.1	60,700	675	1523	562	-4.4	51,900
513	1696	1034	-2.3	29,200	598	1465	1044	-5.0	28,800	676	708	642	-18.8	46,700
514	948	636	-13.2	47,100	599	741	1188	-17.9	23,600	677	919	615	-13.7	48,300
515	481	543	-28.5	53,400	600	907	402	-14.0	68,000	678	1085	551	-10.5	52,700
516	1334	1044	-7.1	28,800	601	687	658	-19.5	45,800	679	600	823	-22.7	33,400
517	868	1021	-14.8	29,700	602	712	1138	-18.7	25,400	680	1237	1004	-8.3	30,300
518	798	779	-16.3	39,600	603	898	181	-14.1	165,200	681	1103	283	-10.1	95,100
519	822	670	-15.7	45,100	604	783	1461	-16.7	14,400	682	1406	477	-6.1	59,100
520	632	165	-21.5	189,000	605	736	223	-18.0	125,300	683	1596	249	-3.4	109,800
521	1332	830	-7.1	37,300	606	629	273	-21.6	98,700	684	555	699	-24.8	43,500
522	603	1104	-22.6	26,600	607	1064	286	-10.8	94,000	685	1167	1313	-9.2	19,300
523	1190	309	-8.9	86,800	608	883	503	-14.5	56,700	686	1932	790	0.0	39,100
524	479	1226	-28.6	22,300	609	2012	610	>0.0	48,700	687	1545	619	-4.1	48,100
525	768	1066	-17.2	28,000	610	1255	903	-8.1	34,200	688	1456	764	-5.2	40,300
526	747	1016	-17.7	29,800	612	1103	391	-10.1	69,600	689	1011	953	-11.8	32,300
527	1170	231	-9.2	119,600	613	778	265	-16.9	102,000	690	1995	270	>0.0	100,200
528	1502	542	-4.6	53,400	614	824	518	-15.7	55,400	691	812	888	-16.0	34,900
529	1728	620	-2.0	48,000	615	1095	195	-10.3	149,100	692	1154	1461	-9.4	14,400
530	507	1011	-27.4	30,000	616	1759	478	-1.6	59,000	693	1993	819	>0.0	37,800
531	870	489	-14.7	57,900	617	994	372	-12.1	72,900	694	1628	656	-3.0	45,900
532	1347	1085	-6.9	27,300	618	751	374	-17.6	72,400	695	928	254	-13.6	107,000
533	1513	346	-4.5	77,800	619	1429	518	-5.7	55,300	696	1854	715	-0.6	42,700
534	308	654	<-35.0	46,000	620	1050	520	-11.1	55,200	697	1997	345	>0.0	78,000
535	1851	689	-0.7	44,100	621	923	1105	-13.7	26,600	698	957	563	-13.0	51,800
536	1463	982	-5.1	31,100	622	1462	622	-5.1	47,900	699	1540	730	-4.2	42,000
537	909	561	-13.9	52,000	623	759	225	-17.4	124,000	702	577	900	-23.8	34,400
538	625	289	-21.7	93,100	624	758	1038	-17.4	29,000	703	1610	562	-3.2	51,900
539	1164	198	-9.2	146,200	625	1438	606	-5.5	48,900	705	1278	571	-7.8	51,200
540	803	655	-16.2	45,900	626	1096	1089	-10.2	27,200	706	1841	704	-0.7	43,300
541	1259	1143	-8.0	25,200	627	942	548	-13.3	53,000	707	1018	1386	-11.7	16,900
542	856	1526	-15.0	12,200	628	809	621	-16.0	48,000	709	1074	1145	-10.7	25,100
543	803	1071	-16.2	27,800	629	899	979	-14.1	31,300	710	293	889	<-35.0	34,800
544	1162	274	-9.3	98,400	630	1135	1321	-9.6	19,100	712	720	412	-18.5	66,600
545	128	1321	<-35.0	19,000	631	979	615	-12.5	48,300	713	1386	841	-6.4	36,800
546	1355	1122	-6.8	25,900	632	1542	1076	-4.1	27,600	714	1328	263	-7.1	103,100
547	595	866	-23.0	35,800	633	1345	814	-6.9	38,000	715	698	433	-19.1	63,900
548	1369	494	-6.6	57,500	634	409	950	-32.2	32,400	716	701	481	-19.0	58,700
549	992	405	-12.2	67,600	635	1165	704	-9.2	43,300	717	1875	699	-0.5	43,600
550	1125	410	-9.8	66,900	636	774	604	-17.0	49,000	718	575	702	-23.9	43,400
551	705	975	-18.9	31,400	637	1263	524	-8.0	54,800	719	1216	204	-8.6	140,400
552	1477	1030	-4.9	29,300	638	952	411	-13.1	66,700	721	1069	484	-10.8	60,400
553	980	583	-12.5	50,400	639	1717	575	-2.1	51,000	722	1272	506	-7.9	56,400
554	700	1109	-19.1	26,400	640	994	292	-12.1	92,000	723	958	822	-13.0	37,700
555	1028	621	-11.5	48,000	641	165	1224	<-35.0	22,400	724	763	395	-17.3	69,100
556	898	794	-14.1	38,900	642	803	251	-16.2	108,900	725	720	916	-18.5	33,700
557	789	1446	-16.6	14,900	643	719	296	-18.5	90,700	726	1476	415	-4.9	66,200
558	777	766	-16.9	40,200	644	1100	294	-10.2	91,400	727	1846	473	-0.7	59,400
559	980	328	-12.5	81,900	645	534	1263	-26.1	21,000	728	510	783	-27.3	39,400
560	1519	611	-4.4	48,600	646	1153	1038	-9.4	29,000	729	1217	1126	-8.6	25,800
561	1212	661	-8.6	45,600	647	1246	204	-8.2	140,000	730	1858	724	-0.6	42,300
562	760	594	-17.4	49,700	648	14	1406	<-35.0	16,200	731	665	765	-20.2	40,300
563	618	956	-21.9	32,100	649	1713	1049	-2.1	28,600	733	1321	312	-7.2	85,900
564	1142	771	-9.6	40,000	650	1986	1183	>0.0	23,800	734	719	427	-18.5	64,600
565	532	787	-26.2	39,300	651	1378	816	-6.5	38,000	735	1101	473	-10.2	59,500
566	771	250	-17.1	109,200	652	1442	1165	-5.5	24,400	736	1359	569	-6.7	51,400
567	1068	534	-10.8	54,100	653	650	806	-20.8	38,400	738	696	220	-19.2	127,600
568	822	734	-15.7	41,800	654	1111	551	-10.0	52,700	739	687	409	-19.5	67,000
569	914	754	-13.8	40,800	655	1095	861	-10.3	36,000	740	1205	256	-8.7	106,200
570	1064	794	-10.8	38,900	656	1524	540	-4.4	53,600	741	995	563	-12.1	51,900
571	1524	714	-4.4	42,800	657	1777	860	-1.4	36,000	742	898	596	-14.1	49,500
572	1392	783	-6.3	39,400	658	391	584	-33.4	50,400	743	881	181	-14.5	165,900
573	982	686	-12.4	44,200	659	977	565	-12.5	51,700	744	1951	686	>0.0	44,200
574	1487	672	-4.8	45,000	660	658	166	-20.5	187,500	745	726	168	-18.3	183,600
575	758	731	-17.4	41,900	661	732	312	-18.1	86,100	746	999	643	-12.0	46,600
576	687	1152	-19.5	24,900	662	1787	567	-1.2	51,500	748	182	1503	<-35.0	13,000
577	930	523	-13.5	55,000	663	888	268	-14.4	100,900	749	2005	649	>0.0	46,300
578	1888	774	-0.4	39,900	664	889	775	-14.3	39,800	750	1448	575	-5.4	51,000
579	642	485	-21.1	58,300	665	715	221	-18.6	126,300	751	792	266	-16.5	101,900
580	1317	519	-7.3	55,300	666	781	227	-16.8	122,400	752	469	296	-28.9	90,600
581	65	1548	<-35.0	11,500	667	646	165	-21.0	189,100	754	664	254	-20.3	107,000
582	1014	614	-11.7	48,400	668	1116	353	-9.9	76,300	755	1195	184	-8.8	161,000
583	732	176	-18.1	172,300	669	1382	643	-6.4	46,600	756	1821	1113	-0.9	26,300
584	1627	478	-3.0	59,000	670	547	789	-25.3	39,200	757	909	246	-13.9	111,000
585	1009	1426	-11.8	15,500	671	984	746	-12.4	41,200	760	790	133	-16.5	264,900

MSN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW	X
761	1399	733	-6.2	41,800	848	1863	271	-0.6	99,500	939	1197	827	-8.8	37,500	405
763	1416	1085	-5.9	27,300	849	1166	523	-9.2	54,900	941	1765	885	-1.5	35,000	1296
764	2020	569	>0.0	51,400	850	1535	1024	-4.2	29,600	942	602	472	-22.7	59,600	856
765	651	475	-20.8	59,300	851	1035	826	-11.4	37,500	943	312	498	<-35.0	57,100	1264
766	1052	1149	-11.1	25,000	852	834	542	-15.5	53,400	944	993	491	-12.1	57,700	986
767	1968	468	>0.0	59,900	855	499	220	-27.8	127,100	945	1300	269	-7.5	100,300	1547
768	1330	685	-7.1	44,300	856	1063	194	-10.9	150,500	946	630	423	-21.6	65,100	1381
769	1970	613	>0.0	48,500	857	887	890	-14.4	34,800	947	187	736	<-35.0	41,600	1525
770	857	617	-15.0	48,200	858	1448	639	-5.4	46,900	948	1380	344	-6.5	78,200	1128
771	1337	974	-7.0	31,500	859	706	311	-18.9	86,200	949	1766	665	-1.5	45,400	1226
773	1576	502	-3.7	56,700	860	1070	1066	-10.7	28,000	950	1038	193	-11.3	151,000	1761
775	969	824	-12.8	37,600	861	472	347	-28.8	77,600	951	860	152	-14.9	213,000	541
776	1438	708	-5.5	43,100	862	674	480	-19.9	58,800	952	957	701	-13.0	43,400	818
777	1539	458	-4.2	61,000	864	1307	499	-7.4	57,000	954	503	547	-27.6	53,000	1036
778	850	434	-15.1	63,800	865	645	887	-21.0	34,900	955	1938	712	>0.0	42,900	1439
779	700	411	-19.1	66,800	866	827	1004	-15.6	30,300	957	1010	816	-11.8	37,900	1540
780	1052	1136	-11.1	25,500	868	685	494	-19.5	57,400	958	768	174	-17.2	174,900	1576
784	1413	529	-6.0	54,400	869	1807	402	-1.0	68,000	960	596	419	-23.0	65,700	1089
785	1364	885	-6.7	35,000	870	1323	783	-7.2	39,400	961	557	409	-24.8	67,100	949
786	1822	835	-0.9	37,100	871	1228	1031	-8.4	29,300	962	887	320	-14.4	83,900	426
787	893	392	-14.3	69,500	872	1904	346	-0.3	77,700	963	564	334	-24.5	80,500	1583
790	616	882	-22.0	35,100	873	556	647	-24.8	46,400	964	969	1155	-12.8	24,800	779
791	451	1429	-29.8	15,400	874	1540	756	-4.2	40,700	965	671	255	-20.0	106,600	1613
792	777	377	-16.9	72,000	875	1566	777	-3.8	39,700	966	1204	798	-8.7	38,700	1380
793	1536	1543	-4.2	11,700	876	1198	351	-8.8	76,800	967	910	154	-13.9	210,300	284
794	1461	807	-5.1	38,300	877	1076	720	-10.6	42,500	968	609	1048	-22.3	28,700	1261
796	388	546	-33.6	53,100	878	1161	1111	-9.3	26,400	969	1285	206	-7.7	138,900	383
797	1126	212	-9.8	133,700	879	647	757	-20.9	40,700	970	822	232	-15.8	119,300	1817
798	933	437	-13.5	63,400	880	1756	594	-1.6	49,700	971	976	437	-12.6	63,400	1245
799	1420	593	-5.9	49,800	881	1543	278	-4.1	97,100	972	403	567	-32.6	51,600	1258
800	1759	279	-1.6	96,500	883	1432	890	-5.7	34,800	974	279	495	<-35.0	57,400	705
801	624	865	-21.7	35,800	884	922	689	-13.7	44,100	975	844	981	-15.3	31,200	1181
802	898	547	-14.2	53,000	885	1103	414	-10.1	66,400	976	1124	295	-9.8	91,100	529
803	1775	1468	-1.4	14,200	886	1501	607	-4.6	48,900	977	994	664	-12.1	45,400	508
804	573	196	-24.0	148,400	887	798	1103	-16.3	26,600	978	1612	642	-3.2	46,700	1898
805	203	494	<-35.0	57,400	888	636	634	-21.3	47,200	979	749	1141	-17.7	25,300	873
806	980	1039	-12.5	29,000	889	951	759	-13.1	40,600	980	1064	642	-10.8	46,700	1768
807	902	308	-14.1	87,200	890	717	548	-18.6	52,900	981	1197	911	-8.8	33,900	836
808	625	827	-21.7	37,500	891	1123	229	-9.8	121,200	983	1762	1508	-1.6	12,800	1863
809	1851	1015	-0.7	29,900	892	891	413	-14.3	66,400	984	1344	317	-6.9	84,700	826
810	440	573	-30.9	51,100	894	1245	234	-8.2	117,800	985	1024	1105	-11.5	26,600	971
811	1358	249	-6.8	109,700	895	1962	346	>0.0	77,700	987	739	1159	-17.9	24,600	1697
812	851	393	-15.1	69,400	896	1322	626	-7.2	47,700	988	816	555	-15.9	52,400	1157
813	745	1246	-17.8	21,600	897	420	570	-31.4	51,300	990	785	361	-16.7	74,900	620
814	2028	810	>0.0	38,200	898	662	428	-20.3	64,500	991	1159	317	-9.3	84,500	1867
815	1086	645	-10.4	46,500	899	845	243	-15.3	113,000	992	1090	928	-10.4	33,300	2019
816	629	313	-21.6	85,700	900	624	703	-21.7	43,400	993	1030	701	-11.5	43,400	1546
817	1376	1177	-6.5	24,000	901	931	1094	-13.5	27,000	994	847	811	-15.2	38,200	1545
818	1771	790	-1.4	39,100	903	799	229	-16.3	121,000	995	902	461	-14.1	60,700	61
819	1045	263	-11.2	103,100	904	765	520	-17.2	55,200	996	888	847	-14.4	36,600	1954
820	984	362	-12.4	74,600	905	775	889	-17.0	34,800	997	1815	579	-0.9	50,700	588
821	1712	279	-2.2	96,700	907	888	824	-14.4	37,600	998	1205	504	-8.7	56,500	1050
822	1256	205	-8.1	139,200	908	828	1303	-15.6	19,700	999	617	289	-22.0	93,100	457
823	1517	654	-4.4	46,000	910	681	1544	-19.7	11,700	1000	968	290	-12.8	92,700	1884
824	1442	449	-5.5	62,000	911	1544	301	-4.1	89,100	1001	970	771	-12.7	40,000	1714
825	1240	513	-8.3	55,800	913	1606	387	-3.3	70,400	1002	1736	478	-1.9	58,900	1717
826	1309	1014	-7.4	29,900	914	1237	688	-8.3	44,100	1003	643	1184	-21.1	23,700	1976
827	2012	708	>0.0	43,100	916	1442	749	-5.5	41,100	1006	822	487	-15.8	58,100	547
828	937	1405	-13.4	16,200	917	1260	367	-8.0	73,700	1007	875	279	-14.6	96,400	1348
830	1342	756	-7.0	40,700	919	764	1541	-17.3	11,700	1009	291	644	<-35.0	46,800	1385
831	562	826	-24.5	37,500	920	1133	1123	-9.7	25,900	1010	1386	745	-6.4	41,200	1078
832	1073	1039	-10.7	29,000	921	1123	380	-9.8	71,500	1011	459	541	-29.4	53,500	975
833	481	820	-28.5	37,800	923	829	242	-15.6	113,200	1012	679	661	-19.7	45,600	1202
834	501	581	-27.8	50,500	924	1131	318	-9.7	84,300	1013	1818	1128	-0.9	25,800	1022
837	751	748	-17.6	41,100	925	1441	874	-5.5	35,400	1014	1032	634	-11.4	47,200	1905
838	635	833	-21.3	37,200	926	679	219	-19.7	128,200	1015	1629	994	-3.0	30,700	1512
839	1494	459	-4.7	60,900	927	1487	1191	-4.8	23,500	1016	1311	1134	-7.4	25,500	1114
840	1952	301	>0.0	89,300	928	1082	775	-10.5	39,800	1017	1722	424	-2.0	65,000	1464
841	1585	1080	-3.6	27,500	929	1231	816	-8.4	38,000	1018	1015	743	-11.7	41,300	1048
842	571	1312	-24.1	19,400	931	1609	670	-3.3	45,100	1020	1574	1219	-3.7	22,500	1122
843	1325	649	-7.2	46,300	932	810	900	-16.0	34,400	1021	781	484	-16.8	58,400	1722
844	1727	301	-2.0	89,200	933	965	520	-12.8	55,100	1022	1129	83	-9.7	591,300	1098
845	630	679	-21.5	44,600	934	947	462	-13.2	60,600	1023	812	317	-15.9	84,800	1830
846	2016	905	>0.0	34,200	936	865	843	-14.8	36,800	1024	785	446	-16.7	62,400	764
847	673	1200	-19.9	23,200	937	1421	1056	-5.9	28,400	1025	1290	739	-7.7	41,500	1968

MSN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW	MSN	X	Y	CPKd	SDSMW
1026	405	552	-32.3	52,600	1153	821	1158	-13.7	24,700	1246	547	577	-25.3	50,800
1027	1298	848	-7.5	36,500	1154	1584	864	-3.5	35,900	1247	530	576	-26.3	50,900
1028	856	547	-15.0	53,000	1161	637	400	-21.3	68,400	1249	516	572	-27.0	51,200
1030	1284	226	-7.7	123,200	1162	623	397	-21.8	68,800	1250	973	536	-12.7	53,800
1031	986	822	-12.3	37,700	1163	665	397	-20.2	68,700	1251	607	532	-22.4	54,200
1032	1547	403	-4.1	67,900	1168	564	528	-24.4	54,500	1252	665	529	-20.2	54,400
1033	1381	551	-6.4	52,700	1170	552	529	-25.0	54,500	1253	899	766	-14.1	40,200
1034	1525	496	-4.3	57,200	1171	538	524	-25.9	54,800	1254	1311	746	-7.4	41,200
1035	1128	645	-8.7	46,500	1172	545	514	-25.5	55,700	1255	1300	761	-7.5	40,400
1036	1226	274	-8.5	98,300	1174	1099	522	-10.2	55,000	1257	1938	712	0.0	42,900
1039	1761	262	-1.6	103,600	1176	1304	586	-7.5	50,200	1258	1806	718	-1.0	42,600
1040	541	839	-25.7	36,900	1177	1366	539	-6.6	53,700	1259	1727	715	-2.0	42,700
1041	818	910	-15.8	34,000	1178	1608	702	-3.3	43,400	1260	1629	713	-3.0	42,800
1044	1036	485	-11.3	58,300	1179	1485	224	-4.8	124,900	1261	1555	717	-4.0	42,600
1045	1439	407	-5.5	67,300	1180	1459	224	-5.2	124,900	1262	1468	717	-5.0	42,600
1047	1540	250	-4.2	108,200	1181	1431	223	-5.7	125,100	1263	1413	722	-6.0	42,400
1048	1576	635	-3.7	47,100	1182	1407	223	-6.1	125,200	1264	1340	717	-7.0	42,600
1049	1089	411	-10.4	66,700	1183	1383	224	-6.4	124,700	1265	1263	717	-8.0	42,600
1050	949	1040	-13.2	28,900	1184	1454	182	-5.3	164,400	1266	1182	720	-9.0	42,500
1051	426	818	-31.1	37,800	1185	1422	183	-5.8	162,600	1267	1110	717	-10.0	42,600
1052	1583	1385	-3.6	16,900	1186	1394	182	-6.3	164,300	1268	1055	717	-11.0	42,600
1053	779	1092	-16.8	27,000	1189	1171	214	-9.2	131,800	1269	999	717	-12.0	42,600
1054	1613	620	-3.2	48,000	1190	1457	286	-5.2	94,200	1270	959	715	-13.0	42,700
1055	1380	377	-6.5	72,000	1191	686	1114	-19.5	26,200	1271	905	712	-14.0	42,900
1056	284	663	<-35.0	45,500	1192	265	893	<-35.0	34,700	1272	857	714	-15.0	42,800
1058	1261	746	-8.0	41,200	1193	403	1292	-32.6	20,000	1273	810	705	-16.0	43,300
1060	393	605	-33.3	49,000	1194	344	1275	<-35.0	20,600	1274	774	711	-17.0	42,900
1061	1817	645	-0.9	46,600	1195	505	1311	-27.6	19,400	1277	737	708	-18.0	43,100
1062	1245	746	-8.2	41,200	1196	572	1293	-24.1	20,000	1278	702	711	-19.0	42,900
1064	1258	792	-8.1	39,000	1197	639	1502	-21.2	13,000	1279	671	710	-20.0	43,000
1065	705	934	-18.9	33,000	1198	637	1402	-21.3	16,300	1280	645	710	-21.0	43,000
1066	1181	734	-9.0	41,800	1199	614	1407	-22.1	16,200	1281	617	707	-22.0	43,100
1067	529	658	-26.3	45,800	1200	637	1431	-21.3	15,400	1282	595	704	-23.0	43,300
1068	508	696	-27.4	43,700	1201	1095	1394	-10.3	16,600	1283	573	700	-24.0	43,500
1069	1898	604	-0.3	49,100	1202	1719	1545	-2.1	11,600	1284	552	695	-25.0	43,700
1071	873	609	-14.7	48,700	1203	791	668	-16.5	45,200	1285	536	694	-26.0	43,800
1073	1768	1128	-1.5	25,800	1204	964	1021	-12.9	29,700	1286	515	687	-27.0	44,200
1075	836	773	-15.4	39,900	1205	313	195	<-35.0	148,700	1287	496	683	-28.0	44,400
1076	1863	861	-0.6	36,000	1208	306	194	<-35.0	149,800	1288	467	669	-29.0	45,200
1078	826	566	-15.7	51,600	1209	320	197	<-35.0	147,400	1289	447	667	-30.9	45,300
1081	971	483	-12.7	58,500	1210	326	197	<-35.0	146,600	1290	427	655	-31.0	45,900
1083	1697	202	-2.3	142,300	1211	394	294	-33.2	91,400	1291	412	655	-32.0	45,900
1085	1157	794	-9.4	38,900	1212	402	294	-32.7	91,200	1292	397	652	-33.0	46,100
1090	620	910	-21.9	34,000	1214	386	294	-33.7	91,400	1293	381	654	-34.0	46,000
1092	1867	597	-0.5	49,500	1215	641	329	-21.2	81,600	1294	365	653	-35.0	46,100
1093	2019	894	>0.0	34,600	1216	660	329	-20.4	81,600	1295	348	653	<-35.0	46,100
1094	1546	538	-4.1	53,700	1217	914	266	-13.8	101,800					
1095	1545	477	-4.1	59,100	1218	873	245	-14.7	112,000					
1098	61	935	<-35.0	33,000	1219	970	372	-12.7	72,900					
1099	1954	237	>0.0	116,000	1220	1021	298	-11.6	90,100					
1101	588	1048	-23.3	28,600	1221	1392	205	-6.3	139,500					
1102	1050	667	-11.1	45,200	1222	1354	203	-6.8	141,800					
1103	457	797	-29.5	38,800	1223	1362	205	-6.7	139,500					
1105	1884	532	-0.4	54,200	1224	673	540	-19.9	53,600					
1106	1714	649	-2.1	46,300	1225	614	542	-22.1	53,400					
1107	1717	546	-2.1	53,100	1226	603	539	-22.6	53,600					
1108	1976	722	>0.0	42,400	1227	696	623	-19.2	47,800					
1111	547	1066	-25.3	28,000	1228	707	628	-18.9	47,500					
1112	1348	621	-6.9	48,000	1229	475	447	-28.7	62,300					
1115	1385	762	-6.4	40,400	1230	466	1282	-29.0	20,400					
1116	1078	816	-10.6	38,000	1231	759	1461	-17.4	14,400					
1117	975	787	-12.6	39,300	1232	1324	1170	-7.2	24,200					
1118	1202	833	-8.7	33,100	1233	1583	1005	-3.6	30,300					
1119	1022	1076	-11.6	27,600	1234	1865	809	-0.6	38,200					
1120	1905	616	-0.3	48,300	1235	1812	817	-1.0	37,900					
1121	1512	1301	-4.5	19,700	1236	1411	703	-6.0	43,400					
1122	1114	677	-9.9	44,700	1237	1392	682	-6.3	44,500					
1123	1464	452	-5.1	61,700	1238	794	410	-16.4	66,900					
1125	1048	857	-11.1	36,200	1239	769	407	-17.1	67,300					
1126	1122	802	-9.8	38,600	1240	740	406	-17.9	67,500					
1128	1722	892	-2.1	34,700	1241	743	511	-17.8	55,900					
1133	1098	825	-10.2	37,500	1242	713	510	-18.7	56,000					
1139	1830	569	-0.8	51,400	1243	682	509	-19.6	56,100					
1147	764	1182	-17.3	23,800	1244	663	504	-20.3	56,500					
1148	1968	724	>0.0	42,300	1245	565	582	-24.4	50,500					

Table 2. Table of some identified proteins

POP name	Protein name	MSN's	Basis for identification
IDS:3_ALPHA_HDDH	3- α -hydroxysteroid-dihydrodiol-dehydrogenase, an enzyme of steroid metabolism	137, 159	Pure protein and antibody provided by Dr. T.M. Penning, Department of Pharmacology, School of Medicine, University of Pennsylvania.
IDS:ACTIN_BETA	β cellular actin, a cytoskeletal protein	38	Homologous position with respect to other mammalian systems
IDS:ACTIN_GAMMA	γ cellular actin, a cytoskeletal protein	68	Homologous position with respect to other mammalian systems
IDS:ALBUMIN	Serum albumin, mature form.	21, 28, 33	Predominance in rat plasma
IDS:APO_A-I	Apo A-I plasma lipoprotein, mature form (lentilae).	236, 463	Presence in rat plasma, regulation by some lipid-lowering drugs
IDS:CALMODULIN	Calmodulin, an acidic cytosolic calcium-binding protein	123, 649	Homologous position with respect to other mammalian systems
IDS:CATALASE	Catalase (peroxisomal)	54, 61, 106	Presence in purified peroxisomes, similarity in position to mouse catalase
IDS:CPKSPOTS	Spots contributed by the CPK charge standards (not rat liver proteins)	1257 - 1295	
IDS:CPS	Carbamoyl phosphate synthase	114, 157, 167, 174, 1184, 1185, 1186, 1222	Pure protein provided by Dr. Margaret Marshall, Department of Pharmacology, Medical School, University of Wisconsin - Madison.
IDS:CYTOCHROME_B5	Cytochrome b5	87, 477	Pure protein provided by Dr. Andrew Parkinson, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center
IDS:FABP-L	Liver fatty-acid binding protein	227	Pure protein provided by Dr. Nathan Bass, Department of Medicine, University of California School of Medicine, San Francisco
IDS:HMG-COA_SYNTHASE	Cytosolic HMG-CoA Synthase	133, 144, 235, 413	Antibody provided by Dr. Michael Greenspan, Merck Sharp & Dohme Research Laboratories, Rahway, NJ
IDS:LAMIN_B	Lamin B, a nuclear protein	415, 734	Homologous position with respect to other mammalian systems
IDS:MITCON:1	Mitcon:1 (F1 ATPase β subunit), a mitochondrial inner membrane	17, 49, 71, 340, 1245, 1246, 1247, 1249	Homologous position with respect to other mammalian systems, presence in mitochondria
IDS:MITCON:2	Mitcon:2, a mitochondrial matrix stress protein equivalent to E.	15, 25, 110, 1241, 1242, 1243, 1244	Homologous position with respect to other mammalian systems, presence in mitochondria
IDS:MITCON:3	Mitcon:3, a mitochondrial matrix stress protein, likely analog of	18, 35, 226, 600, 1238, 1239, 1240	Homologous position with respect to other mammalian systems, presence in mitochondria
IDS:NADPH_P450_RED	NADPH cytochrome P-450 reductase, frequently co-induced with P-450's	175, 251, 812	Pure protein provided by Dr. Andrew Parkinson, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center
IDS:PD1	Protein disulphide isomerase 1	168, 1170, 1171, 1172	Sequence information obtained by R.M. Van Frank, Lilly Research Laboratories, Indianapolis
IDS:PLASMA_PROTEINS	Rat plasma proteins observed in liver	21, 28, 33, 44, 72, 102, 115, 197, 236, 246, 248, 257, 293, 332, 347, 364, 369, 419, 432, 463, 468, 518, 562, 605, 623, 666, 667, 725, 738, 790, 865, 903, 926	Plasma coelectrophoresis studies
IDS:PRO-ALBUMIN	Serum albumin precursor	47, 93	Relative position to mature albumin, presence in microsome
IDS:PYRCARBOX	Pyruvate carboxylase	179, 1180, 1181, 1182, 1183	Pavlica, R.J., et al., BBA (1990) 1022 115-125.
IDS:SOD	Superoxide dismutase	135	Sequence information obtained by R.M. Van Frank, Lilly Research Laboratories, Indianapolis
IDS:TUBULIN_ALPHA	α tubulin, a cytoskeletal protein	56, 132, 1224, 1252	Homologous position with respect to other mammalian systems
IDS:TUBULIN_BETA	β tubulin, a cytoskeletal protein	50, 1225, 1226, 1251	Homologous position with respect to other mammalian systems

Computed:
hemoglobin

Protein

Rabbit r

Hb-beta

3. Computed pI's of two sets of carbamylated protein standards: Rabbit muscle CPK and human hemoglobin (Hb)

Protein Name	PIR Name	#ASP 3.9	#GLU 4.1	#HIS 6.0	#LYS 10.8	#ARG 12.5	NH2- 7.0	Calc pI	Real CPK
Rabbit muscle CPK	KIRBCM	28	27	17	34	18	1	6.84	0.0
		28	27	17	33	18	1	6.67	-1
		28	27	17	32	18	1	6.54	-2
		28	27	17	31	18	1	6.42	-3
		28	27	17	30	18	1	6.31	-4
		28	27	17	29	18	1	6.21	-5
		28	27	17	28	18	1	6.12	-6
		28	27	17	27	18	1	6.03	-7
		28	27	17	26	18	1	5.94	-8
		28	27	17	25	18	1	5.85	-9
		26	27	17	24	18	1	5.76	-10
		28	27	17	23	18	1	5.67	-11
		28	27	17	22	18	1	5.58	-12
		28	27	17	21	18	1	5.48	-13
		28	27	17	20	18	1	5.39	-14
		28	27	17	19	18	1	5.29	-15
		28	27	17	18	18	1	5.20	-16
		28	27	17	17	18	1	5.12	-17
		28	27	17	16	18	1	5.04	-18
		28	27	17	15	18	1	4.96	-19
		28	27	17	14	18	1	4.89	-20
		28	27	17	13	18	1	4.83	-21
		28	27	17	12	18	1	4.77	-22
		28	27	17	11	18	1	4.71	-23
		28	27	17	10	18	1	4.66	-24
		28	27	17	9	18	1	4.61	-25
		28	27	17	8	18	1	4.56	-26
		28	27	17	7	18	1	4.52	-27
		28	27	17	6	18	1	4.48	-28
		28	27	17	5	18	1	4.44	-29
		28	27	17	4	18	1	4.40	-30
		28	27	17	3	18	1	4.36	-31
		28	27	17	2	18	1	4.32	-32
		28	27	17	1	18	1	4.29	-33
		28	27	17	0	18	1	4.25	-34
		28	27	17	0	18	0	4.22	-35
Hb-beta, human	HBHU	7	8	9	11	3	1	7.18	
		7	8	9	10	3	1	6.79	
		7	8	9	9	3	1	6.53	-1.8
		7	8	9	8	3	1	6.32	-3.2
		7	8	9	7	3	1	6.13	-5.3
		7	8	9	6	3	1	5.96	-7.2
		7	8	9	5	3	1	5.78	-10.0
		7	8	9	4	3	1	5.59	-12.3
		7	8	9	3	3	1	5.37	-15.5
		7	8	9	2	3	1	5.14	-18.0
		7	8	9	1	3	1	4.91	-21.0
		7	8	9	0	3	1	4.71	-25.5
		7	8	9	0	3	0	4.54	-27.2

Table 4. Computed pI's of some known proteins related to measured CPK pI's

Protein Name	PIR Name	#ASP 3.9	#GLU 4.1	#HIS 6.0	#LYS 10.8	#ARG 12.5	Calc pI	Real CPK
0 Creatine phospho kinase (CPK), rabbit muscle	KIRBCM	28	27	17	34	18	6.84	0.0
1 Fatty acid-binding protein, rat hepatic	FZRTL	5	13	2	16	2	7.83	-3.0
2 b2-microglobulin, human	MGHUB2	7	8	4	8	5	6.09	-5.0
3 Carbamoyl-phosphate synthase, rat	SYRTCA	72	96	28	95	56	5.97	-5.5
4 Proalbumin (serum albumin precursor), rat	ABRTS	32	57	15	53	27	5.98	-6.2
5 Serum albumin, rat	ABRTS	32	57	15	53	24	5.71	-9.0
6 Superoxid dismutase (Cu-Zn, SOD), rat	A26810	8	11	10	9	4	5.91	-9.2
7 Phospholipase C, phosphoinositide-specific (?), rat	A28807	34	42	9	49	21	5.92	-9.2
8 Albumin, human	ABHUS	36	61	16	60	24	5.70	-11.9
9 Apo A-I lipoprotein, rat	A24700	18	24	6	23	12	5.32	-13.7
10 proApo A-I lipoprotein, human	LPHUA1	16	30	6	21	17	5.35	-14.3
11 NADPH cytochrome P-450 reductase, rat	RDRTO4	41	60	21	38	36	5.07	-15.6
12 Retinol binding protein, human	VAHU	18	10	2	10	14	5.04	-16.9
13 Actin beta, rat	ATRTC	23	26	9	19	18	5.06	-17.2
14 Actin gamma, rat	ATRTC	20	29	9	19	18	5.07	-16.8
15 Apo A-I lipoprotein, human	LPHUA1	16	30	5	21	16	5.10	-17.5
16 Apo A-IV lipoprotein, human	LPHUA4	20	49	8	28	24	4.88	-19.7
17 Tubulin alpha, rat	UBRTA	27	37	13	19	21	4.66	-19.8
18 F1ATPase beta, bovine	PWBOB	25	36	9	22	22	4.80	-21.0
19 Tubulin beta, pig	UBPGB	26	36	10	15	22	4.49	-22.5
20 Protein disulphide isomerase (PDI), rat hepatic	ISRTSS	43	51	11	51	9	4.07	-25.0
21 Cytochrome b5, rat	CBRT5	10	15	6	10	4	4.59	-26.0
22 Apo C-II lipoprotein, human	LPHUC2	4	7	0	6	1	4.44	-30.5
Amino acid pI assumed in calculation:		3.9	4.1	6.0	10.8	12.5		

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An updated two-dimensional gel database of rat liver proteins useful in gene regulation and drug effect studies

We have improved upon the reference two-dimensional (2-D) electrophoretic map of rat liver proteins originally published in 1991 (N. L. Anderson *et al.*, *Electrophoresis* 1991, 12, 907-930). A total of 53 proteins (102 spots) are now identified, many by microsequencing. In most cases, spots cut from wet, Coomassie Blue stained 2-D gels were submitted to internal tryptic digestion [2], and individual peptides, separated by high-performance liquid chromatography (HPLC), were sequenced using a Perkin-Elmer 477A sequencer. Additional spots were identified using specific antibodies.

Figure 1 shows the current annotated 2-D map of F344 rat liver, analyzed using the Iso-DALT system (20 × 25 cm gels) and BDH 4-8 carrier ampholytes. Both the map itself and the master spot number system remain the same as shown in the original publication. Table 1 lists the important features of each identification shown, including the gel position, *pI*, and *M_r*, for the most abundant or most basic form of each protein. Using this extended base of identified spots, a series of four improved calibration functions has been derived for the *pI* and SDS-*M_r* axes (the first two of which are shown in Fig. 2A and B). Both forward and reverse functions are derived, so that one can compute the physical properties of a spot with a given gel location, or inversely compute the gel position expected for a protein having given physical properties:

$$Y_{\text{RATLIVER}} = f_{M-\text{RATLIVER}}(M_{\text{SEQUENCE-DERIVED}}) \quad (1)$$

$$X_{\text{RATLIVER}} = f_{pI-\text{RATLIVER}}(pI_{\text{SEQUENCE-DERIVED}}) \quad (2)$$

$$M_{\text{GEL-DERIVED}} = f_{\text{RATLIVER } Y-M_r}(Y_{\text{RATLIVER}}) \quad (3)$$

$$pI_{\text{GEL-DERIVED}} = f_{\text{RATLIVER } X-pI}(X_{\text{RATLIVER}}) \quad (4)$$

A spreadsheet program (in Microsoft Excel) was developed to facilitate flexible computation of *pI*'s from amino acid sequence data, and the results were entered into a relational database (Microsoft Access). A table of spot positions and sequence-derived *pI*'s and *M_r*'s was fitted with a large series of analytic equations using Tablecurve (Jandel Scientific), and the four conversion Eqs. (1)-(4), relating computed *pI* and gel *X* coordinate, or computed molecular weight and gel *Y* coordinate, were selected, based on criteria of simplicity, goodness of fit and favorable asymptotic behavior. Table 2 lists the equations and coefficients. Application of Eqs. (3) and (4) to a spot's *X* and *Y* coordinates, given in [1], produce improved *M_r* estimates, and allow computation of *pI*

directly in *pH* units, instead of in terms of positions relative to creatine phosphokinase (CPK) charge standards. The inverse Eqs. (1) and (2) were used to compute the gel positions of a series of *pI* and *M_r* tick marks. These tick marks were plotted with SigmaPlot (Jandel), together with fiducial marks locating several prominent spots, and the resulting graphic was aligned over the synthetic gel image (computed by Kepler from the master gel pattern) using Freelance (Lotus Development). Maps were printed as Postscript output from Freelance, either in black and white (as shown here) or in color, where label color indicates subcellular location (available from the first author upon request). We have also used the rat liver 2-D pattern as presented here to calibrate the patterns of other samples. Using mixtures of rat liver and mouse liver samples, for example, we made composite 2-D patterns that allow use of the rat pattern to standardize both axes of the mouse pattern. This was accomplished by deriving transformations relating the rat and mouse *X*, and separately the rat and mouse *Y*, axes (Table 2, lower half; Fig. 2C and D) based on a series of spots that coelectrophorese in these closely related species. These functions were then applied to derive equations relating the mouse liver *X* and *Y* to *pI* and SDS-*M_r* (Eqs. 5 and 6 below). The resulting standardized 2-D pattern for B6C3F1 mouse liver is shown in Fig. 3.

$$M_{\text{MOUSELIVER}} = f_{\text{RATLIVER } Y-M_r}(M_{\text{MOUSELIVER } Y-\text{RATLIVER } Y} (Y_{\text{MOUSELIVER}})) \quad (5)$$

$$pI_{\text{MOUSELIVER}} = f_{\text{RATLIVER } X-pI}(pI_{\text{MOUSELIVER } X-\text{RATLIVER } X} (X_{\text{MOUSELIVER}})) \quad (6)$$

A slightly more complex approach can be used to standardize samples that have few or no spots co-electrophoresing with rat liver proteins. In this case, a 2-D gel is prepared with a mixture of the two samples, and four functions (forward and backward, each for *X* and *Y*) are derived relating each sample's own master pattern to the composite. The required functions are then applied in a nested fashion to yield the desired result (using rat plasma as an example):

$$M_{\text{RATPLASMA}} = f_{\text{RATLIVER } Y-M_r}(f_{\text{RATPLASMA } Y-\text{RATLIVER } Y}(f_{\text{RATPLASMA } Y-\text{RATPLASMA } Y}(Y_{\text{RATPLASMA}}))) \quad (7)$$

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Keywords: Two-dimensional polyacrylamide gel electrophoresis / Liver / Map / Identification / Calibration

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Table 1. continued

MSN ^{a)}	Protein IDb)	Protein name	Identification comments	Gel X ^{a)}	Experimental pI ^{a)}	Gel Y ^{a)}	Experimental M _r ^{a)}
1184, 1186, 114, 174, 118 5, 167, 157 54, 61	CPSM_RAT	Carbamyl phosphate synthase	2-D of pure protein; confirmed by N-terminal sequence and AAA	1453.56	6.05	181.64	160 640
136	CATA_RAT	Catalase	Internal sequence	2000.81	6.73	499.64	58 968
	COX2_RAT	COX-II	Ab (J. W. Taanman), confirmed by internal sequence	452.57	4.61	1062.67	25 504
87	CYB5_RAT	Cytochrome B5	2-D of pure protein; Ab; confirmed by AAA	515.68	4.73	1370.55	18 493
41	CK-RAT ^{a)}	Cytokeratin	Location in cytoskeletal fraction	1165.12	5.75	569.09	51 448
29	CK-RAT ^{a)}	Cytokeratin	Location in cytoskeletal fraction	743.11	5.15	605.23	48 187
5, 11	ENPL-RAT ^{a)}	Endoplasmic	Ab (F. Witzmann)	567.73	4.83	263.37	112 194
60	ENOA_RAT	Enolase A	Internal sequence and AAA	1399.78	6.00	623.54	46 674
27	ER60_RAT	ER-60	N-Terminal sequence (R. M. Van Frank)	1184.20	5.77	523.51	56 169
17	ATPB_RAT	F1 ATPase β	N-Terminal sequence and AAA	629.06	4.95	588.83	49 620
196	ATP7_RAT	F1 ATPase δ	Internal sequence	1227.24	5.82	1184.65	22 310
79	F16P_RAT	Fructose-1,6-bis-phosphatase	Uncertain; by comparison with ID in Garrison and Wager (JBC 257:13135-13143)	924.54	5.44	737.77	38 858
62, 78	DHE3_RAT	Glutamate dehydrogenase	N-Terminal sequence and internal sequence	1887.39	6.55	566.92	51 655
125	HAST-RAT ^{a)}	HAST-I: N-hydroxyaryl-amine sulfoxtransferase	Internal sequence	1297.94	5.89	861.55	32 638
307	HO1_RAT	Heme oxygenase 1	Uncertain; available data from internal sequence	1219.39	5.81	915.71	30 423
413, 1250, 933	HMCS_RAT	HMG CoA synthase, cytosolic	Ab (J. Gernershausen)	1033.48	5.59	538.13	54 571
133, 144, 235	HMCS_RAT	HMG CoA synthase, mitochondrial (frag)	Ab (J. Gernershausen), N-terminal sequence (Steiner/Lotuspeich)	666.40	5.02	1019.42	26 811
8, 23, 1307	HS7C_RAT	HSC-70	Positional homology (with human, etc.) through coelectrophoresis	811.87	5.27	425.76	69 521
15, 25, 110	P60_RAT	HSP-60	Ab (F. Witzman); confirmed by N-terminal sequence and AAA	845.09	5.32	520.03	56 561
971	HS70-RAT ^{a)}	HSP-70	Ab (F. Witzman)	976.11	5.51	437.14	67 674
1216, 1215, 90	HS90-RAT ^{a)}	HSP-90	Ab (F. Witzman)	659.86	5.00	329	90 107
256	ING1-HUMAN	Interferon- γ induced protein	Internal sequence	993.85	5.54	1006.04	27 237
415, 734	LAMB-RAT ^{a)}	Lamin B	Positional homology with human through coelectrophoresis, nuclear location	737.10	5.14	425.19	69 615
80	LAMR-RAT ^{a)}	"Laminin receptor"	Internal sequence	534.02	4.77	697.62	41 327
227	FABL_RAT	L-FABP (liver fatty acid binding protein)	Ab (N. M. Bass)	1586.09	6.18	1483.43	16 622
134	MDHC_MOUSE E	Malate dehydrogenase	Internal sequence	1270.85	5.86	861.96	32 620
18, 35, 226	GR75-RAT ^{a)}	Mitcon-3; grp75	Positional homology with human through coelectrophoresis	905.67	5.41	413.67	71 589
175, 251	NCPR_RAT	NADPH P450 reductase	2-D of pure protein	824.69	5.29	393.21	75 366
1168, 1170, 1171	PDI_RAT	PDI: Protein disulfide isomerase	N-Terminal sequence (R. M. van Frank), Ab	564.30	4.83	528.47	55 618
47, 93	ALBU_RAT	Pro-Albumin	Microsomal lumen location, pI, M _r relative to albumin	1391.03	5.99	446.68	66 195
236	APA1_RAT	Pro-APO A-I lipoprotein	Coelectrophoresis with plasma protein	920.41	5.43	1137.51	23 467
320	IPK1_BOVIN	Protein kinase C inhibitor 1	Internal sequence; homology with bovine protein	1480.01	6.08	1458.81	17 007
152	PNPH_MOUSE	Purine nucleoside phosphorylase	Internal sequence	1507.19	6.10	911.16	30 599
1179, 1180, 1181, 1182, 1183	PYVC-RAT ^{a)}	Pyruvate carboxylase	Tentative; 2-D of pure protein (J. G. Henslee, JBC, 1979); reported in <i>Biochim. Biophys. Acta</i> 1022, 115-125	1485.10	6.08	223.52	131 589
55, 103	SM30_RAT	SMP-30: Senescence marker protein-30	Internal sequence	721.71	5.11	830.10	34 051
135	SODC_RAT	Superoxide dismutase	AAA; confirmed by internal sequence (R. M. Van Frank)	1161.24	5.74	1388.68	18 173
172	TPM-RAT ^{a)}	Tm: tropomyosin	Location in cytoskeleton, 2-D position relative to human, Ab	476.24	4.66	957.86	28 865
277, 56	TBA1_RAT	Tubulin α	Positional homology with human through coelectrophoresis, cytoskeletal location	688.22	5.06	537.67	54 620
50, 1225	TBB1_RAT	Tubulin β	Positional homology with human through coelectrophoresis, cytoskeletal location	621.29	4.93	535.48	54 855
1224	VIME_RAT	Vimentin	Positional homology with human through coelectrophoresis, cytoskeletal location	673.00	5.03	539.50	54 426

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B6C3F1 MOUSE LIVER 2-D PROTEIN PATTERN

v1.1 (B6C3F1MST2) 28-Apr-1995 © by Large Scale Biology Corporation,
1620 Medical Center Drive, Rockville, MD 20850 USA 301/424-9099
MW and computed pI scales are shown to known proteins

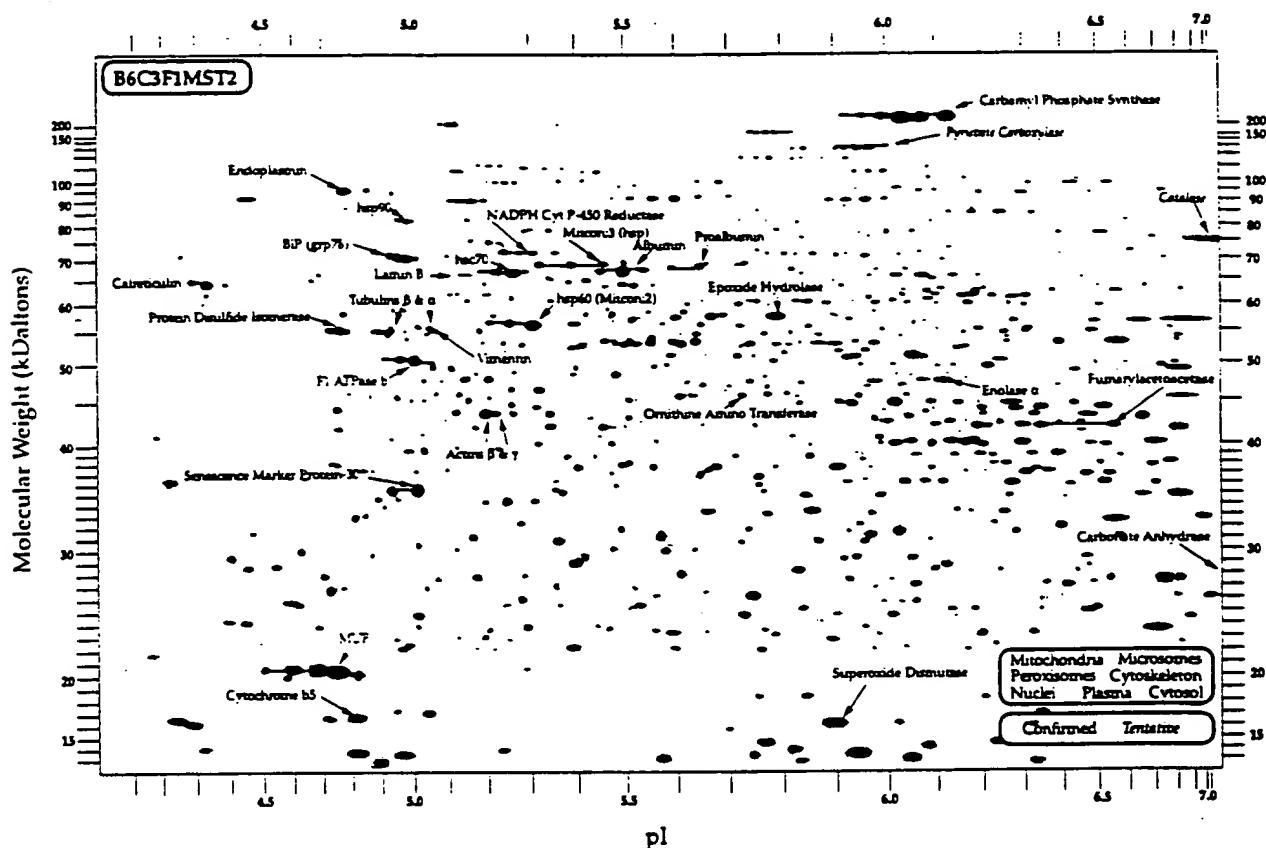


Figure 3. Master 2-D gel pattern for B6C3F1 mouse liver, standardized using the F344 rat liver pattern identifications, according to the method described in the text. Twenty-nine proteins are identified.

$$pI_{\text{RATPLASMA}} = \frac{f_{\text{RATLIVER}} \times pI_{\text{RATPLASMA-LIVER}} \times f_{\text{RATLIVER}}}{f_{\text{RATPLASMA}} \times f_{\text{RATPLASMA-LIVER}} \times f_{\text{RATPLASMA}}} \quad (8)$$

This unified approach, in which one well-populated 2-D pattern is used to standardize a family of other patterns, has the additional advantage that the resulting pI and M_r scales are directly compatible. Hence one can compare the relative pI 's of mouse and rat versions of a sequenced protein in a consistent pI measurement system, and select likely inter-species analogs based on positional relationships on common scales. Adoption of immobilized pH gradient (IPG) technology [4-7] will result in substantial improvements in pI positional reproducibility for standard 2-D maps such as those presented here; however, we believe that our approach will continue to be useful in establishing the empirical pH gradient actually achieved by such gels under given experimental conditions (temperature, urea concentration, etc.), in relating patterns run on different IPG ranges and using different lots of IPG gels (between which some variation will persist). Development of rodent organ maps is a continuing effort in our laboratories [8-10], and results in regular additions of identified proteins. Those who wish to receive current rodent liver maps, with color annotations, should send a stamped self-addressed envelope to the first author.

We would like to thank the individuals who provided antibodies mentioned in Table 1, and R. M. van Frank for unpublished sequenced data.

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Progress with Proteome Projects: Why all Proteins Expressed by a Genome Should be Identified and How To Do It

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Introduction

The advent of large genome sequencing projects has changed the scale of biology. Over a relatively short period of time, we have witnessed the elucidation of the complete nucleotide sequence for bacteriophage λ (Sanger *et al.*, 1982), the nucleotide sequence of an eukaryotic chromosome (Oliver *et al.*, 1992), and in the near future will see the definition of all open reading frames of some simple organisms, including *Mycoplasma pneumoniae*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Cuonorbabditis elegans* and *Arabidopsis thaliana*. Nevertheless, genome sequencing projects are not an end in themselves. In fact, they only represent a starting point to understanding the function of an organism. A great challenge that biologists now face is how the co-expression of thousands of genes can best be examined under physiological and pathophysiological conditions, and how these patterns of expression define an organism.

There are two approaches that can be used to examine gene expression on a large scale. One uses nucleic acid-based technology, the other protein-based technology. The most promising nucleic acid based technology is differential display of mRNA (Liang and Pardee, 1992; Bauer *et al.*, 1993), which uses polymerase chain reaction with arbitrary primers to generate thousands of cDNA species, each which correspond to an expressed gene or part of a gene. However, it is currently unclear if this technique can be developed to reliably assay the expression of thousands of genes or

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identify all cDNA species, and the approach does not easily allow a systematic screening. Analysis of gene expression by the study of proteins present in a cell or tissue presents a favorable alternative. This can be achieved by use of two-dimensional (2-D) gel electrophoresis, quantitative computer image analysis, and protein identification techniques to create 'reference maps' of all detectable proteins. Such reference maps establish patterns of normal and abnormal gene expression in the organism, and allow the examination of some post-translational protein modifications which are functionally important for many proteins. It is possible to screen proteins systematically from reference maps to establish their identities.

To define protein-based gene expression analysis, the concept of the 'proteome' was recently proposed (Wilkins *et al.*, 1995; Wasinger *et al.*, 1995). A proteome is the entire PROTEin complement expressed by a genOME, or by a cell or tissue type. The concept of the proteome has some differences from that of the genome, as while there is only one definitive genome of an organism, the proteome is an entity which can change under different conditions, and can be dissimilar in different tissues of a single organism. A proteome nevertheless remains a direct product of a genome. Interestingly, the number of proteins in a proteome can exceed the number of genes present, as protein products expressed by alternative gene splicing or with different post-translational modifications are observed as separate molecules on a 2-D gel. As an extrapolation of the concept of the 'genome project', a 'proteome project' is research which seeks to identify and characterise the proteins present in a cell or tissue and define their patterns of expression.

Proteome projects present challenges of a similar magnitude to that of genome projects. Technically, the 2-D gel electrophoresis must be reproducible and of high resolution, allowing the separation and detection of the thousands of proteins in a cell. Low copy number proteins should be detectable. There should be computer gel image analysis systems that can qualitatively and quantitatively catalog the electrophoretically separated proteins, to form reference maps. A range of rapid and reliable techniques must be available for the identification and characterisation of proteins. As a consequence of a proteome project, protein databases must be assembled that contain reference information about proteins; such databases must be linked to genomic databases and protein reference maps. Databases should be widely accessible and easy to use.

Recently, there have been many changes in the techniques and resources available for the analysis of proteomes. It is the aim of this chapter to discuss the status of the areas outlined above, and to review briefly the progress of some current proteome projects.

Two-dimensional electrophoresis of proteomes

Two dimensional (2-D) gel electrophoresis involves the separation of proteins by their isoelectric point in the first dimension, then separation according to molecular weight by sodium dodecyl sulfate electrophoresis in the second dimension. Since first described (Klose, 1975; O'Farrell, 1975; Scheele, 1975), it has become the method of choice for the separation of complex mixtures of proteins, albeit with many modifications to the original techniques. 2-D electrophoresis forms the basis of proteome projects through separating proteins by their size and charge (Hochstrasser *et al.*,

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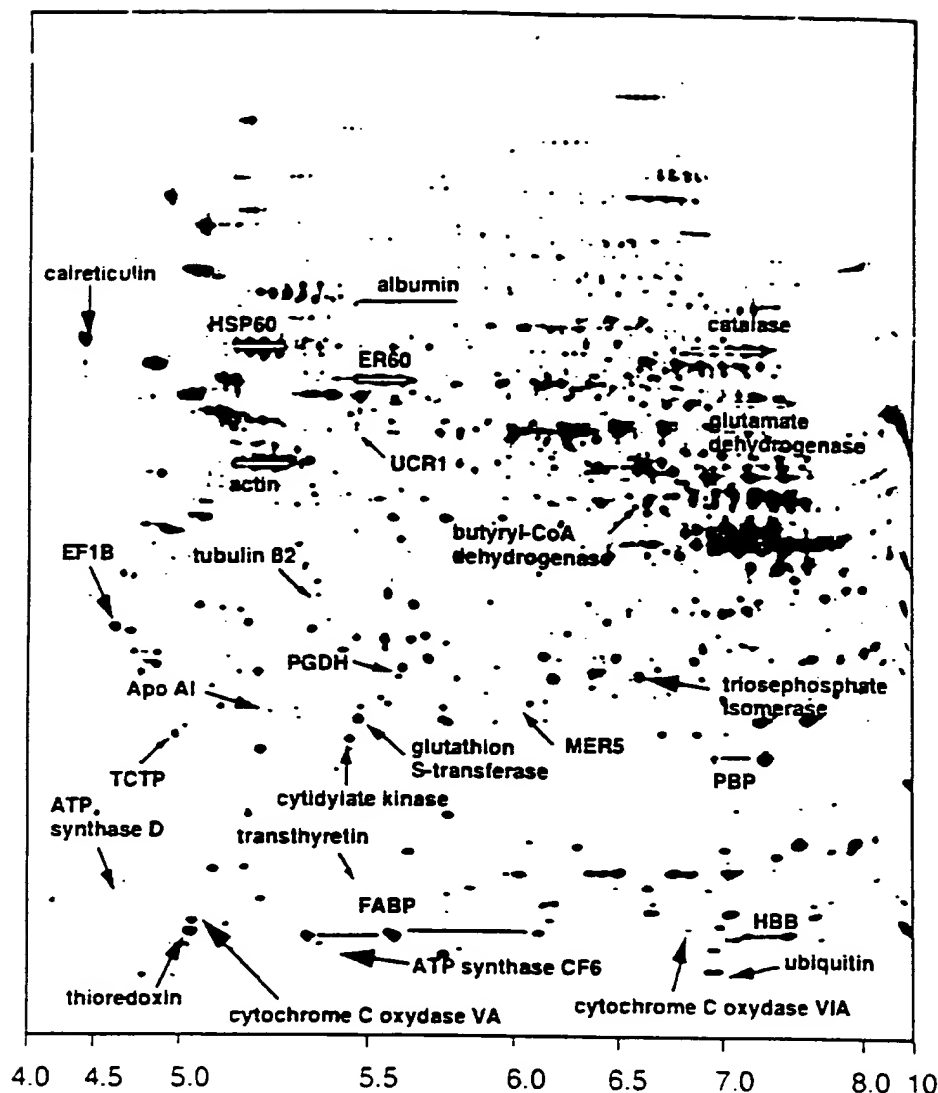


Figure 1. Two-dimensional gel electrophoresis map of a human hepatoblastoma-derived cell line, illustrating the very high resolution of the technique. The first dimensional separation (right to left of figure) was achieved using immobilised pH gradient electrophoresis of 4.0 to 10.0 units. The second dimension (top to bottom of figure) was SDS-PAGE using a 11%–14% acrylamide gradient, allowing separation in the molecular weight range 10–250 kDa. Proteins were visualised by silver staining. Arrows show proteins of known identity.

1992; Celis *et al.*, 1993; Garrels and Franza, 1989; VanBogelen *et al.*, 1992). Current protocols can resolve two to three thousand proteins from a complex sample on a single gel (Figure 1).

2-D GEL RESOLUTION AND REPRODUCIBILITY

A primary challenge of separating complex mixtures of proteins by 2-D gel electrophoresis has been to achieve high resolution and reproducibility. High resolution ensures that a maximum of protein species are separated, and high reproducibility is

vital to allow comparison of gels from day to day and between research sites. These factors can be difficult to achieve.

Carrier ampholytes are a common means of isoelectric focusing for the first dimension of 2-D electrophoresis. Gels are usually focused to equilibrium to separate proteins in the pI range 4 to 8, and run in a non-equilibrium mode (NEPHGE) to separate proteins of higher pI (7 to 11.5) (O'Farrell, 1975; O'Farrell, Goodman and O'Farrell, 1977). Unfortunately, the use of carrier ampholytes in the isoelectric focusing procedure is susceptible to 'cathode drift', whereby pH gradients established by prefocusing of ampholytes slowly change with time (Righetti and Drysdale, 1973). Carrier ampholyte pH gradients are also distorted by high salt concentration of samples (Bjellqvist *et al.*, 1982), and by high protein load (O'Farrell, 1975). A further limitation is that isoelectric focusing gels, which are cast and subject to electrophoresis in narrow glass tubes, need to be extruded by mechanical means before application to the second dimension – a procedure that potentially distorts the gel. Nevertheless, many of the above shortcomings can be avoided by loading small amounts of ^{14}C or ^{35}S radiolabelled samples (Garrels, 1989; Neidhardt *et al.*, 1989; Vandekerckhove *et al.*, 1990). High sensitivity detection is then achieved through use of fluorography or phosphorimaging plates (Bonner and Laskey, 1974; Johnston, Pickett and Barker, 1990; Patterson and Latter, 1993). However, this approach is only practicable for organisms or tissues that can be radiolabelled.

An alternative technique, which is becoming the method of choice for the first dimension separation of proteins, involves isoelectric focusing in immobilized pH gradient (IPG) gels (Bjellqvist *et al.*, 1982; Görg, Postel and Gunther, 1988; Righetti, 1990). Immobilized pH gradients are formed by the covalent coupling of the pH gradient into an acrylamide matrix, creating a gradient that is completely stable with time. IPG gels are usually poured onto a stiff backing film, which is mechanically strong and provides easy gel handling (Ostergren, Eriksson and Bjellqvist, 1988). The major advantages of IPG separations are that they do not suffer from cathodic drift, they allow focusing of basic and very acidic proteins to equilibrium, pH gradients can be precisely tailored (linear, stepwise, sigmoidal), and that separations over a very narrow pH range are possible (0.05 pH units per cm) (Righetti, 1990; Bjellqvist *et al.*, 1982, 1993a; Sinha *et al.*, 1990; Görg *et al.*, 1988; Gelfi *et al.*, 1987; Gunther *et al.*, 1988). However, it is not currently possible to use IPG gels to separate very basic proteins of isoelectric point greater than 10, although this is under development. Narrow pH range separations are useful to address problems of protein co-migration in complex samples, allowing 'zooming in' on regions of a gel (Figure 2). IPG gel strips are now commercially available, which begin to address the problems of intra- and inter-lab isoelectric focusing reproducibility.

There are two means of electrophoresis for the second dimension separation of proteins: vertical slab gels and horizontal ultrathin gels (Görg, Postel, and Gunther, 1988). Both are usually SDS-containing gradient gels of approximately 11% to 15% acrylamide, which separate proteins in the molecular mass range of 10 – 150 kD. A stacking gel is not usually used with slab gels, but is necessary when using horizontal gel setups (Görg, Postel and Gunther, 1988). Comparisons have shown that there is little or no difference in the reproducibility of electrophoresis using either approach (Corbett *et al.*, 1994a), but commercially available vertical or horizontal precast gels will provide greater reproducibility for occasional users. For slab gel electrophoresis,

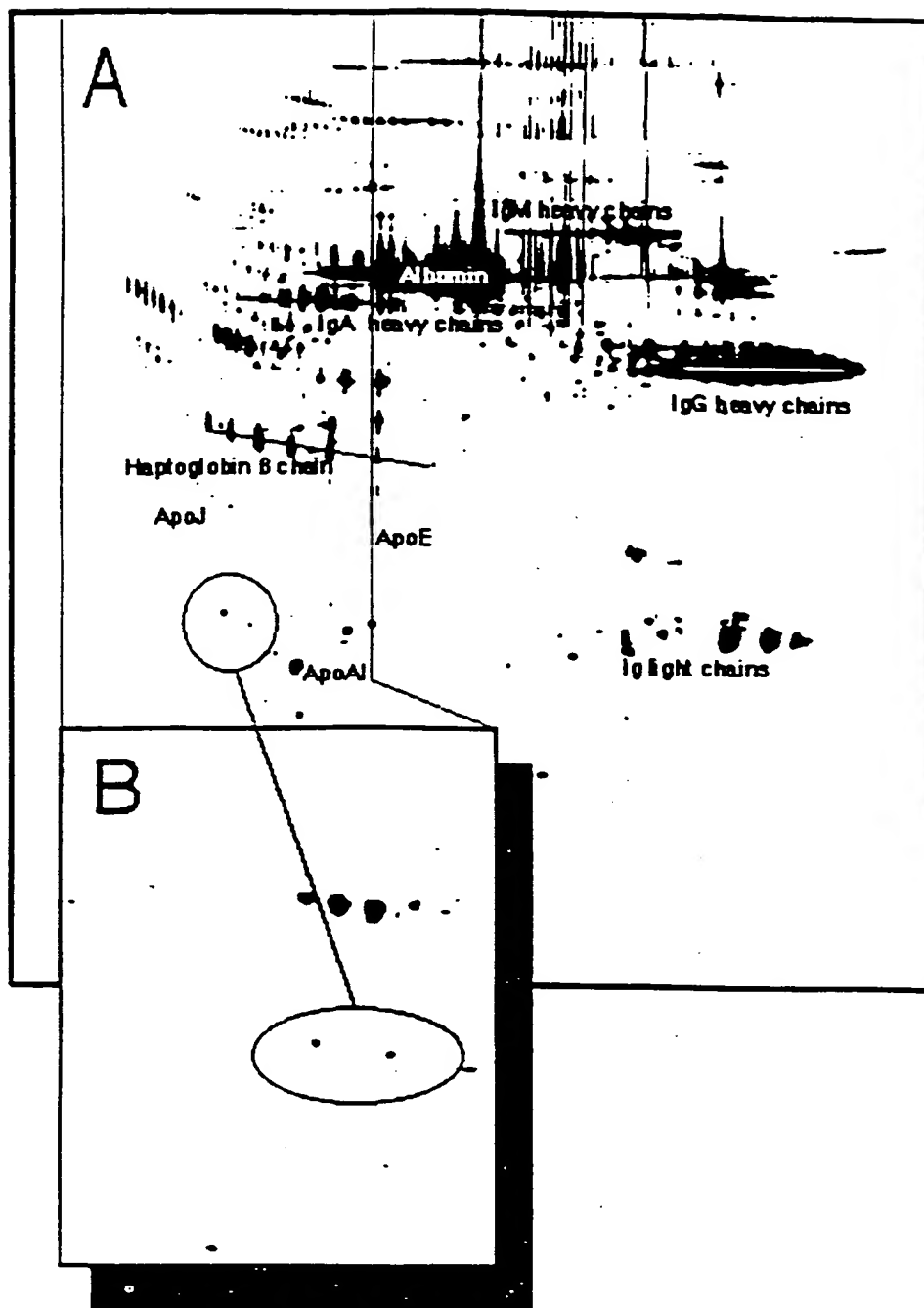


Figure 2. Two-dimensional gel electrophoresis allows 'zooming in' on areas of interest. Rings highlight 2 proteins common to each gel. (A) Wide pI range two dimensional electrophoresis map of human plasma proteins. First dimension separation was achieved using an immobilised pH gradient of 3.5 to 10.0 units. The second dimension was SDS-PAGE. Actual gel size was 16cm x 20cm, and proteins were visualised with silver staining. (B) Narrow pI range electrophoresis was used to 'zoom in' on a small region of the plasma map. The first dimension used a narrow range immobilised pH gradient of 4.2 to 5.2 units, and second dimension was SDS-PAGE. Micropreparative loading was used, and the gel blotted to PVDF. Proteins were visualised with amido black. Actual blot size was 16cm x 20cm.

the use of piperazine diacrylyl as a gel crosslinker and the addition of thiosulfate in the catalyst system has been shown to give better resolution and higher sensitivity detection (Hochstrasser and Merril, 1988; Hochstrasser, Patchornik and Merril, 1988).

Notwithstanding the advances described above, there is an increasing demand to improve the reproducibility of 2-D electrophoresis to facilitate database construction and proteome studies. Harrington *et al.* (1993) explain that if a gel resolves 4000 protein spots, and there is 99.5% spot matching from gel to gel, this will produce 20 spot errors per gel. This amount of error, which might accumulate with each gel to gel comparison used in database construction, could produce an unacceptable degree of uncertainty in gel databases. To address these issues, partial automation of large 2-D gel separations has been undertaken (Nokihara, Morita and Kuriki, 1992; Harrington *et al.*, 1993). Although results are preliminary, spot to spot positional reproducibility in one study was found to be threefold improved over manual methods (Harrington *et al.*, 1993). It should be noted that small 2-D gel formats (50 × 43 mm) have been almost completely automated (Brewer *et al.*, 1986), although these are not generally used for database studies.

MICROPREPARATIVE 2-D GEL ELECTROPHORESIS

With the advent of affordable protein microcharacterisation techniques, including N-terminal microsequencing, amino acid analysis, peptide mass fingerprinting, phosphate analysis and monosaccharide compositional analysis, a new challenge for 2-D electrophoresis has been to maintain high resolution and reproducibility but to provide protein in sufficient quantities for chemical analysis (high nanogram to low microgram quantities of proteins per spot). This becomes difficult to achieve with very complex samples such as whole bacterial cells, as the initial protein load is divided among 2000 to 4000 protein species. Two approaches are used for producing amounts of material that can be chemically characterised. The first method is to run multiple gels, collect and pool the spots of interest, and subject them to concentration (Ji *et al.*, 1994; Walsh *et al.*, 1995; Rasmussen *et al.*, 1992). In this approach, the concentration process must also act as a purification step to remove accumulated electrophoretic contaminants such as glycine. A more elegant approach has been to exploit the high loading capacity of IPG isoelectric focusing. The high loading capacity of immobilised pH gradients was described early (Ek, Bjellqvist and Righetti, 1983), but has only recently been applied to 2-D electrophoresis (Hanash *et al.*, 1991; Bjellqvist *et al.*, 1993b). Up to 15 mg of protein can be applied to a single gel, yielding microgram quantities of hundreds of protein species. A further benefit of this approach is that proteins present in low abundance, which may not be visualised by lower protein loads, are more likely to be detected. The use of electrophoretic or chromatographic prefractionation techniques (Hochstrasser *et al.*, 1991a; Harrington *et al.*, 1992), followed by high loading of narrow-range IPG separations (Bjellqvist *et al.*, 1993b) provides a likely solution to studies on proteins present in low abundance.

Methods of protein detection

There are many means for detecting proteins from 2-D gels. The method used will be dictated by factors including protein load on gel (analytical or preparative), the purpose of the gel (for protein quantitation or for blotting and chemical characterisation), and the sensitivity required. The most common means of protein detection and their applications are shown in *Table 1*. Most detection methods have drawbacks, for

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Table 1: Common stains for 2-D gels or blots and their applications.

Detection Method	Main applications	Unsuitable applications	Sensitivity	References
[³⁵ S] Met or ¹⁴ C radiolabelling and fluorography or phosphorimaging	Cell lines, cultured organisms	Samples that cannot be labelled	20 ppm of radiolabel in a spot	Gariels and Franza, 1989; Latham, Garret's and Solter, 1993
[³⁵ S]thiourea silver	Extremely high sensitivity gel staining	Preparative 2-D; PVDF or NC membranes	0.4 ng protein on spot or band of gel	Wallace and Saluz, 1992a,b
Silver	Very high sensitivity gel staining, can be mono or polychromatic	Preparative 2-D; PVDF or NC membranes	4 ng protein on spot or band of gel	Rabilloud, 1992; Hochstrasser and Merrill, 1988
Coomassie blue R-250	Staining of gels; staining of PVDF membranes before protein sequencing	Staining prior to direct mass determination from PVDF; amino acid analysis on PVDF; detection of some glycoproteins	40 ng protein on band or spot of gel	Strupat <i>et al.</i> , 1994; Gharahdaghi <i>et al.</i> , 1992; Goldberg <i>et al.</i> , 1988; Sanchez <i>et al.</i> , 1992
Colloidal gold	Staining NC membranes, staining PVDF before direct MALDI-TOF	Gels	60 × higher than coomassie	Yamaguchi and Asakawa, 1988; Eckerskorn <i>et al.</i> , 1992; Strupat <i>et al.</i> , 1994
Zinc imidazole	Reverse staining of gels or membranes; may be beneficial in MALDI-TOF of peptides	Where positive image is required	Higher than coomassie	Ortiz <i>et al.</i> , 1992; James <i>et al.</i> , 1993
Ponceau S and amido black	Staining higher protein loads on PVDF, for protein sequencing or amino acid analysis	Staining prior to direct mass determination from PVDF	100 ng protein on band or spot of gel	Sanchez <i>et al.</i> , 1992; Strupat <i>et al.</i> , 1994; Wilkins <i>et al.</i> , 1995
India ink	Staining of membrane-bound proteins; staining PVDF before direct MALDI-TOF	Gel staining; not quantitative from protein to protein	1–10 ng	Li <i>et al.</i> , 1989; Hughes, Mack and Hamparian, 1988; Strupat <i>et al.</i> , 1994
Stains-all	Staining to detect glycoproteins or Ca ²⁺ binding proteins	General gel staining	100 ng protein on band or spot of gel	Campbell, MacLennan and Jorgensen, 1983; Goldberg <i>et al.</i> , 1988

PVDF = polyvinylidene difluoride, NC = nitrocellulose, MALDI-TOF = matrix assisted laser desorption ionisation time of flight mass spectrometry.

example, some glycoproteins are not stained by coomassie blue (Goldberg *et al.*, 1988), and many organic dyes are unsuitable for protein detection on PVDF if samples are to be used for direct matrix-assisted laser desorption ionisation mass spectrometry (Strupat *et al.*, 1994).

Although most means of protein detection give some indication of the quantities of protein present, in general they cannot be used for global quantitation. This is because

no protein, stain is able consistently to detect proteins over a wide range of concentrations, isoelectric points and amino acid compositions, and with a variety of post-translational modifications (Goldberg *et al.*, 1988; Li *et al.*, 1989). Furthermore, there are large differences in staining pattern when identical gels or blots are subjected to different stains, including amido black, imidazole zinc, india ink, ponceau S, colloidal gold, or coomassie blue (Tovey, Ford and Baldo, 1987; Ortiz *et al.*, 1992). The most common means of quantitating large numbers of proteins in a 2-D gel involves the radiolabelling of protein samples prior to electrophoresis, and protein quantitation based on fluorography and image analysis or liquid scintillation counting (Garrels, 1989; Celis and Olsen, 1994). However, proteins which do not contain methionine cannot be detected if only [35 S] methionine is used for labelling. Amino acid analysis of protein spots visualised by other techniques presents a likely means of protein quantitation for the future.

BLOTTING OF PROTEINS TO MEMBRANES

Electrophoretic blotting of proteins from two-dimensional polyacrylamide gels to membranes presents many options for protein identification and microcharacterisation which are not possible when proteins remain in gels. For example, when proteins are blotted to polyvinylidene difluoride (PVDF) membranes, they can be identified by N-terminal sequencing, amino acid analysis, or immunoblotting, or they may be subjected to endoproteinase digestion, monosaccharide analysis, phosphate analysis, or direct matrix-assisted laser desorption ionisation mass spectrometry (Matsudaira, 1987; Wilkins *et al.*, 1995; Jungblut *et al.*, 1994; Sutton *et al.*, 1995; Rasmussen *et al.*, 1994; Weizthandler *et al.*, 1993; Murthy and Iqbal, 1991; Eckerskorn *et al.*, 1992). It is possible to combine some of these procedures on a single protein spot on a PVDF membrane (Packer *et al.*, 1995; Wilkins *et al.*, submitted; Weizthandler *et al.*, 1993). This is useful when minimal amounts of protein are available for analysis. These techniques will be explored in detail later in this review. Notwithstanding the above, there are some disadvantages associated with blotting of proteins to membranes. There is always loss of sample during blotting procedures (Eckerskorn and Lottspeich, 1993), and common protein detection methods are less sensitive or not applicable to membranes (Table 1), presenting difficulties for the analysis of low abundance proteins. Detailed discussion of the merits of available membranes and common blotting techniques can be found elsewhere (Eckerskorn and Lottspeich, 1993; Strupat *et al.*, 1994; Patterson, 1994).

2-D gel analysis, documentation, and proteome databases

Following protein electrophoresis and detection, detailed analysis of gel images is undertaken with computer systems. For proteome projects, the aim of this analysis is to catalogue all spots from the 2-D gel in a qualitative and if possible quantitative manner, so as to define the number of proteins present and their levels of expression. Reference gel images, constructed from one or more gels, form the basis of two-dimensional gel databases. These databases also contain protein spot identities and

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